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The Fe^{2+}-Mediated Decomposition, PfATP6 Binding, and Antimalarial Activities of Artemisone and Other Artemisinins: The Unlikelihood of C-Centered Radicals as Bioactive Intermediates

Richard K. Haynes,*[a] Wing Chi Chan,[a] Chung-Man Lung,[a] Anne-Catrin Uhlemann,[b] Ursula Eckstein,[b] Donatella Taramelli,[c] Silvia Parapini,[c] Diego Monti,[d] and Sanjeev Krishna*[b]

The results of Fe^{2+}-induced decomposition of the clinically used artemisinins, artemisone, other aminoartemisinins, 10-deoxoartemisinin, and the 4-fluorophenyl derivative have been compared with their antimalarial activities and their ability to inhibit the parasite SERCA PfATP6. The clinical artemisinins and artemisone decompose under aqueous conditions to give mixtures of C radical marker products, carbonyl compounds, and reduction products. The 4-fluorophenyl derivative and aminoartemisinins tend to be inert to aqueous iron(II) sulfate and anhydrous iron(II) acetate. Anhydrous iron(II) bromide enhances formation of the carbonyl compounds and provides a deoxyglycal from DHA and enamines from the aminoartemisinins. Ascorbic acid (AA) accelerates the aqueous Fe^{2+}-mediated decompositions, but does not alter product distribution. 4-Oxo-TEMPO intercepts C radicals from a mixture of an antimalaria-active trioxolane, 10-deoxoartemisinin, and anhydrous iron(II) acetate to give trapped products in 73% yield from the trioxolane, and 3% from the artemisinin. Artemisone provides a trapped product in 10% yield. Thus, in line with its structural rigidity, only the trioxolane provides a C radical eminently suited for intermolecular trapping. In contrast, the structural flexibility of the C radicals from the artemisinins allows facile extrusion of Fe^{2+} and collapse to benign isomerization products. The propensity towards the formation of radical marker products and intermolecular radical trapping have no relationship with the in vitro antimalarial activities of the artemisinins and trioxolane. Desferrioxamine (DFO) attenuates inhibition of PfATP6 by, and antagonizes antimalarial activity of, the aqueous Fe^{2+}-susceptible artemisinins, but has no overt effect on the aqueous Fe^{2+}-inert artemisinins. It is concluded that the C radicals cannot be responsible for antimalarial activity and that the Fe^{2+}-susceptible artemisinins may be competitively decomposed in aqueous extra- and intracellular compartments by labile Fe^{2+}, resulting in some attenuation of their antimalarial activities. Interpretations of the roles of DFO and AA in modulating antimalarial activities of the artemisinins, and a comparison with antimalarial properties of simple hydroperoxides and their behavior towards thapsigargin-sensitive SERCA ATPases are presented. The general basis for the exceptional antimalarial activities of artemisinins in relation to the intrinsic activity of the peroxide within the uniquely stressed environment of the malaria parasite is thereby adumbrated.

Introduction

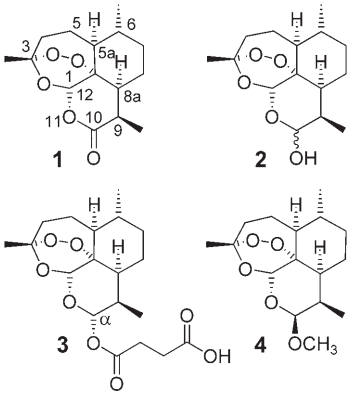
The isolation and discovery of the antimalarial activity of artemisinin **1** represents one of the great events in medicine in the latter half of the 20th Century.[1] Artemisinin and its derivatives dihydroartemisinin (DHA, **2**), artesunate **3**, and artemether **4**[2–4] are now widely used for treatment of malaria caused by the most virulent parasite, *Plasmodium falciparum*.[5] The pivotal problem in their use, that of a short pharmacological half-life, was uncovered by the Chinese and countered early on by administering artemisinin in combination with the pharmacologically robust mefloquine.[6] Consequently, artemisinins are now routinely used in combination with longer half-life drugs,[7] and emphasis is now placed on the development of fixed combinations.

[a] *Prof. Dr. R. K. Haynes, W. C. Chan, C.-M. Lung*
Department of Chemistry, Open Laboratory of Chemical Biology
Institute of Molecular Technology for Drug Discovery and Synthesis
The Hong Kong University of Science and Technology
Clear Water Bay, Kowloon, Hong Kong (PR China)
Fax: (+852) 2358-1594
E-mail: haynes@ust.hk

[b] *Dr. A.-C. Uhlemann, Dr. U. Eckstein, Prof. Dr. S. Krishna*
Centre for Infection, Division of Cellular and Molecular Medicine
St. George's Hospital, University of London SW17 0RE (UK)
E-mail: s.krishna@sgul.ac.uk

[c] *Prof. Dr. D. Taramelli, S. Parapini*
Dipartimento di Salute Pubblica-Microbiologia-Virologia
Università di Milano, Via Pascal 36, 20133 Milan (Italy)

[d] *Prof. Dr. D. Monti*
Institute of Science and Molecular Technology (ISTM)
Via Venezian 21, 20133 Milan (Italy)



In contrast to the universal acceptance of the utility of artemisinins as the most potent and rapidly acting of all antimalarial drugs, a seemingly intractable controversy surrounds the manner in which these drugs are presumed to act as parasiticidal agents. It has been proposed that activity arises as a result of reductive or Fenton-like cleavage of the peroxide by Fe^{2+} to generate alkoxyl radicals, which either abstract hydrogen atoms from the artemisinin periphery or undergo β-scission to provide carbon-centered radicals (C radicals) (Figure 1). The C radicals are held to alkylate proteins vital to parasite function.[8–13] Formation of C radical intermediates in laboratory experiments is demonstrated by interception with thiols[10] or spin-trapping agents.[9,14] C Radicals are purportedly involved in the alkylation of the heme when ferrous heme is used to induce cleavage of the peroxide in artemisinin and analogues.[15,16] In the absence of trapping, the radical intermediates proceed to form stable end products, such as **14** and **15** from artemisinin (Figure 1 and below),[9,17,18] which thereby serve as markers for the intercession of such radicals. The products are at the same oxidation level as the starting artemisinin; the Fe^{2+} cation acts as a catalyst, as it is regenerated during their formation.[9] However, Fe^{2+} is considered to engage in a second-order reaction with artemisinin under aqueous conditions.[19] The results nevertheless are better interpreted in terms of a catalytic reaction, first-order in artemisinin, being accelerated by the excess of the catalyst.

Whilst an artemisinin–heme interaction in one form or another within the food vacuole of the malaria parasite has been held responsible for antimalarial activity,[15,16] the 'heme theory' itself is contentious. Artemisinins are vigorous inducers of CYP enzymes[20] in which the iron in the heme prosthetic group must cycle through the ferrous state in order to induce hydroxylation of the periphery of the molecule *syn* to the peroxide. This leaves the peroxide intact, as we first pointed out in 2001.[21] According to model studies, treatment of artemisinin with ferrous heme generates an artemisinin C radical, which undergoes an exceedingly rapid alkylation of the heme (of the order of a bond vibration).[22] As ligand exchange is required to free the putative C radical from the heme iron,[23] it cannot migrate away from the heme, and exclusively provides a heme–artemisinin adduct. Whilst the latter possesses antimalarial activity, this tends to be in the micromolar range in in vitro assays.[15,24] It has been suggested that the exogenously applied adduct may not readily permeate the vacuolar membrane into the food vacuole.[15] Thus, this relatively weak activity may be ascribed to the redox properties of the ferric heme nucleus within the adduct enhancing oxidative stress within the cytosol of the parasite, as discussed below. Current data tend to indicate that the site of action of artemisinins is not within the food vacuole,[25] and it is established that artemisinins do not inhibit hemozoin formation.[26] However, assertions that artemisinins or artemisinin–heme adducts exert their effects by inhibiting hemozoin formation,[24] thereby allowing buildup of 'toxic' heme monomer in the food vacuole, or that heme is both the 'activating agent' and 'the target' of artemisinin continue to appear.[15,16] The detection of radiolabeled artemisinin within artemisinin–heme adducts in minor amounts in urine or spleen extracts of infected mice administered with an excess of artemisinin,[27] whilst indicating unambiguous although minor sequestration of artemisinins by heme, are indicative of routes of excretion of metabolites, and cannot be taken at all as evidence of mechanism. Indeed, it is recognized that competitive sequestration of artemisinins by heme, as takes place in the foregoing experiment, attenuates their antimalarial activity.[28]

The idea that iron is required for activation of artemisinins appears to rely in large measure on reports that iron chelators such as desferrioxamine B (DFO) antagonize the antimalarial activities of artemisinins in vitro.[29,30] DFO is a hydrophilic chelating agent with exceptional affinity for Fe^{3+}. The Fe^{3+} chelate ferrioxamine B (FO) possesses a stability constant of 10^{31}, which is 10^{24} times greater than that of the Fe^{2+} chelate.[31] Notably, DFO is parasiticidal at a concentration of ~15 μM against *P. falciparum*, a level which is about 60-fold lower than that required for a cytostatic effect against mammalian cells. DFO affects late trophozoites and early schizonts, whereas artemisinins are effective parasiticidal agents against the early ring stage of the parasite. The antagonism cannot be reproduced

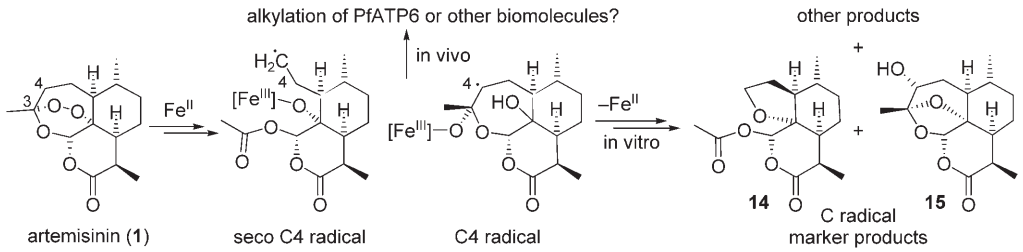


Figure 1. Fe^{2+}-catalysed conversion of artemisinin **1** into "radical marker" products via seco C4 and C4 radicals held to be responsible for biological activity.

in vivo.[32] Antagonism in vitro may arise by removal of the bioavailable or 'labile' iron (as Fe^{3+}) required for parasite metabolism[33] or other effects.[34] FO is reduced by glutathione or ascorbic acid under aqueous conditions, providing that an Fe^{2+} chelating agent is present.[35] DFO and FO markedly modulate intracellular damage caused by peroxides,[36] DFO acts as a free radical scavenger for hydroxyl and peroxyl radicals[37] and abolishes the in vivo antimalarial activity of alloxan, a non-peroxidic compound.[38] In relation to the basis of the antagonistic affect of iron chelators towards artemisinin, it was earlier assumed that iron chelators bind directly to heme to suppress activation of the artemisinin.[15,29] As ferric heme is inert towards artemisinin[17] and DFO displays exquisite specificity for Fe^{3+}, the proposals that DFO associates with the ferrous heme required for 'activation' of the artemisinin and that DFO 'abstracts' Fe^{2+} from the heme[15] are implausible. An alternative explanation proffered was that the alkylated heme adduct "tends to release free iron".[24] Release of iron from the adducts under physiological conditions has not been demonstrated; the adducts are stable, requiring forcing conditions to remove the iron.[39] The inability of DFO to abstract Fe^{3+} from hematin has been demonstrated,[40] and DFO has no effect on artemisinin–heme inhibition of erythrocyte membrane-bound ATPase activity.[41]

Our own survey on the decomposition of artemisinin **1** with heme Fe^{2+}, free Fe^{2+} including Fe^{2+} generated by reduction of Fe^{3+} in situ with cysteine, in aqueous and nonaqueous solutions[17] coupled with a consideration of structure–activity relationships among artemisinin analogues, and the evident inability of certain active antimalarial cyclic peroxides to generate C radicals caused us to reject their feasibility as biologically significant intermediates.[13,17,21,42] More recently, we identified inconsistencies between the reaction of artemisinins with ferrous heme and their decomposition by free Fe^{2+} under aqueous conditions.[43] Our work was criticized on the grounds that some of the artemisinins, because of their presumed insolubility in the aqueous medium, cannot interact with the iron.[44] It was demonstrated that one of our aqueous Fe^{2+}-inert artemisinins efficiently alkylated ferrous heme under aprotic conditions, which are drastically different to those in the food vacuole of the parasite, the presumed site of "heme activation" of the artemisinins.[23] However, 10-deoxoartemisinin **5** (Figure 2), which displays greater antimalarial activity than artemisinin, is rapidly decomposed by aqueous Fe^{2+}, but not by aqueous ferrous heme,[23] and it has no effect on hemozoin formation.[26]

Artemisinins, like the sesquiterpene thapsigargin (TG), are inhibitors of the malaria parasite sarco-/endoplasmic reticulum Ca^{2+} (SERCA) ATPase.[45] Inhibition of SERCA orthologues of *P. falciparum* (PfATP6), *P. vivax* (PvSERCA), and *P. berghei* (PbSERCA) by artemisinins correlates with their ability to kill these parasites.[46] Specificity appears to be associated with residues lining the TG binding cleft of SERCAs, because altering a single amino acid in transmembrane helix 3 through an L263E mutation in PfATP6 decreases sensitivity to artemisinins.[46] That a single substitution elsewhere in the PfATP6 sequence segregates with the phenotype of increased IC_{50} for artemether in parasites from patients would appear to corroborate the assignment of PfATP6 as a target for the artemisinins.[47] Artemisone **7** (Figure 2),[48] a representative of the class of 10-amino-10-dihydroartemisinins,[43] in phase II trials cures malaria patients at one third the dose of the clinically used artesunate **3**.[49] Other aminoartemisinins such as **8** and **9** express exceptional activities in vivo against *P. berghei* in mice (sc ED_{90} values: **8**, 0.6 mg kg^{-1}; **9**, 0.35 mg kg^{-1}; artesunate **3**, 7.2 mg kg^{-1}).[48] Artemisone is an especially effective inhibitor of parasite SERCAs: K_i PfATP6 **7**, 1.7 ± 0.6 nM (artemisinin **1**, 169 ± 31 nM); K_i PvSERCA **7**, 0.072 ± 0.012 nM (**1**, 7.7 ± 4.9 nM).[46] Therefore, according to the Fe^{2+}-activation model, artemisone and the other aminoartemisinins are required to generate C radicals which are especially adept at mediating their biological activities, presumably by alkylating the ATP6 site.

Thus, the advent of artemisone, with its well-defined physicochemical properties coupled with both its potent antimalarial activity and inhibition of PfATP6, provides a robust opportunity to probe the relationship between its Fe^{2+}-mediated decomposition to putative C radicals and biological properties. 4-Oxo-TEMPO efficiently reacts with radicals formed during Fe^{2+}-mediated decomposition of synthetic trioxolanes; the results indeed have been used as a basis for design of new trioxolane antimalarials.[50,51] Therefore, it is expedient to scrutinize this construct by evaluating the ability of 4-oxo-TEMPO to trap the putative C radicals from artemisone and other artemisinin derivatives. We also examined the effects of DFO on PfATP6 inhibition and antimalarial activity of the artemisinins of Figure 2, and the effect of ascorbic acid on the aqueous Fe^{2+}-mediated decomposition of the artemisinin derivatives. Ascorbic acid antagonizes in vitro antimalarial activity of the clinically used artemisinins,[30,52] but it is also a potent reductant for Fe^{3+} to Fe^{2+} in aqueous solutions, even where the former is associated with normally stable Fe^{3+} chelates.[35,53]

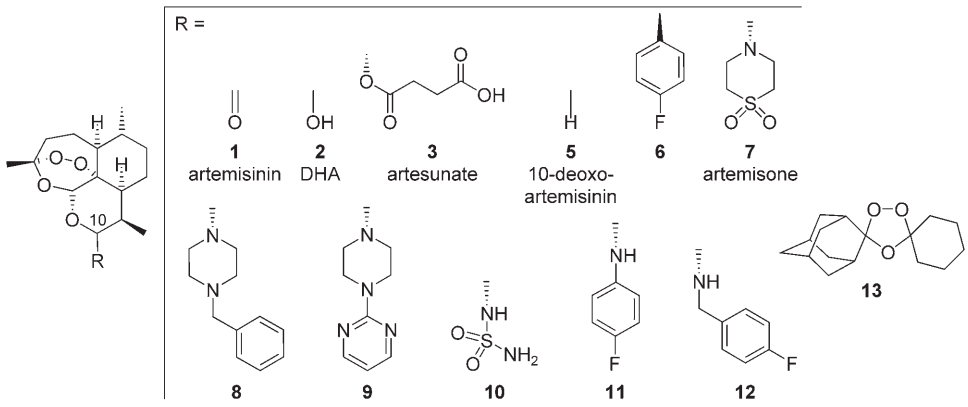


Figure 2. Artemisinins **1**–**12** and synthetic trioxolane **13**[51] used in this study.

Results

a. Ferrous iron mediated decomposition

i. Artemisinins 1–3

The decomposition of artemisinin **1**, DHA **2**, and artesunate **3** by ferrous sulfate in 1:1 acetonitrile/water at 20 °C during 24 h (method a), although examined elsewhere,[9,17] was repeated herein under milder conditions in order to provide a comparison of results with our artemisinins. Structures of products and yields, including literature data from similar reactions conducted at 37 °C (method al),[9] are given in Table 1.

Ascorbic acid (method b) slightly enhanced the decomposition rates; reaction half-lives (Table 1) were determined from plots of decomposition of artemisinin as a function of time. Artemisinins **1** and **7** (below) were inert to an excess of ascorbic acid in the absence of iron. An accelerating affect of ascorbic acid on aqueous Fe^{2+}-catalyzed reactions has not been reported previously, although its presence apparently results in the formation of minor amounts of other products.[18] DHA **2** with $FeBr_2$ in THF (method c)[8–10,54] gave not the radical marker products **16** and **17**,[9] but rather the tricarbonyl compound **18** as a single epimer and the 2-deoxyglycal **19**. At room temperature, **18** underwent conversion into a mixture containing 25% of a second epimer. The same mixture was obtained by thermolysis of DHA at 100 °C.[55] A mixture of **18** together with an isomer described as the C6 epimer was originally obtained by thermolysis of DHA at 190 °C.[56] However, epimerization is shown to occur at C1′ and not at C6.[55] The identity of **19** was secured by dehydration of 2-deoxyDHA[2] with methanesulfonyl chloride and triethylamine in dichloromethane. Ferrous acetate in 1:1 acetonitrile/dichloromethane (method d), which effectively induces decomposition of synthetic trioxolanes such as **13**,[50,51] had little effect on DHA; approximately 72% was recovered, together with small amounts of the tricarbonyl compound **18** and the furan **20**.

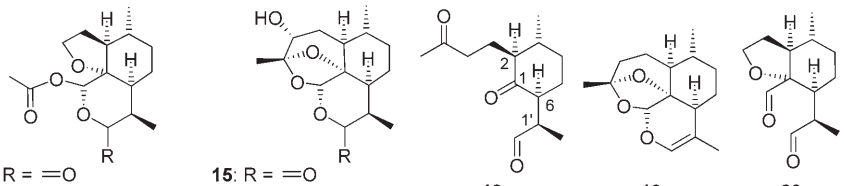
ii. Artemisinins 5 and 6

Exposure of artemisinins **5** and **6** to ferrous sulfate/ acetonitrile/water gave the products and their yields as shown in Table 2. Whereas **5** was efficiently decomposed, **6** is essentially inert. Ascorbic acid accelerated decomposition of the former, but had no effect on the latter. The tricarbonyl compound **26**, a single epimer, emerges as the dominant product from **5** at the expense of the radical marker products **24** and **25** with $FeBr_2$/THF. The reagent has also been reported previously to provide **26** and **28** from **5** (method cl, Table 2).[54] The tricarbonyl compound **26** was obtained as the sole product by heating **5** at 100 °C (method e, Table 2). Compound **27**, obtained previously from **5** by a rearrangement driven by activation of the peroxide bridge by complexation with a Lewis acid,[57] likely arises in the present case via a similar event triggered by Fe^{2+} or Fe^{3+}. Ferrous acetate in 1:1 acetonitrile/dichloromethane gave the radical marker product **24** and the tricarbonyl compound **26**.

iii. 10-Amino-10-dihydroartemisinins 7–12

With the exception of artemisone **7**, the aminoartemisinins tended to be relatively inert under aqueous conditions (Table 3). However, ascorbic acid enhanced the decomposition of the benzylpiperazine derivative **8** and had a slight effect on the pyrimidylpiperazine derivative **9**. With $FeBr_2$/THF, the derivatives gave complex product mixtures from which only relatively small amounts of discrete decomposition products could be isolated. The products include 2-deoxyDHA **35** from **8**, tricarbonyl compound **18**, sulfamide ($H_2NSO_2NH_2$) from **10**, and the enamines **38** and **40** from the primary amino derivatives **10** and **11**. Identification of the enamines follows from a consideration of their ^1H and ^{13}C NMR data, the conversion of **38** by ruthenium trichloride/sodium periodate into the amide **41**, and identification by spectroscopic methods (Experimental Section). The enamines **38** and

Table 1. Yields of products obtained from Fe^{2+}-mediated decomposition of artemisinins **1**–**3**.



14: R = =O
16: R = OH
22: R = $OCO(CH_2)_2COOH$

15: R = =O
17: R = OH
23: R = $OCO(CH_2)_2COOH$

18

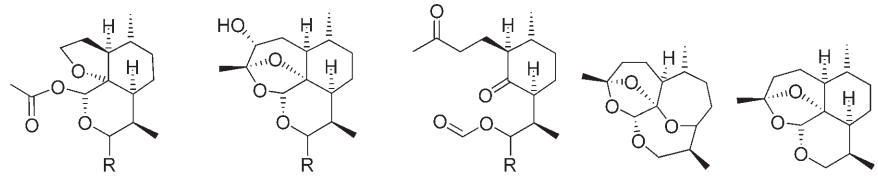
19

20

Starting Artemisinin R Group (Figure 2)	Method[a]	Artemisinin Remaining and Products [%]	Reaction Half-Life [h]
1: artemisinin	a	**1**: 31, **14**: 20, **15**: 26	15.4
R=O	al	**1**: 0, **14**: 25, **15**: 67	
	b[b]	**1**: 11, **14**: 32, **15**: 32	7.3
2: DHA	al	**2**: 0, **16**: 39, **17**: 56	
R=–OH	c	**2**: 0, **18**: 57, **19**: 2	
	d	**2**: 72, **18**: 4, **20**: 12	
3: artesunate	a	**3**: 6, **22**: 46, **23**: 22	6.3
R=–$OOC(CH_2)_2COOH$	al	**3**: 0, **17**: 25, **22**: 45, **23**: 23	
	b	**3**: 2, **22**: 56, **23**: 22	4.3

[a] Method a) artemisinin (0.2–0.4 mmol), $FeSO_4$ (0.3 equiv), MeCN/H_2O (1:1), N_2, 20 °C, 24 h; method al) from Ref. [9]: artemisinin (2 mmol), $FeSO_4$ (2 mmol), MeCN/H_2O (1:1), N_2, 37 °C, 24 h; method b) as for method a) + ascorbic acid (AA, 1.0 equiv with respect to artemisinin); method c) artemisinin (0.8–1.0 mm), $FeBr_2$ (0.5 equiv), THF, N_2, 20 °C, 45 min; method d) $Fe(OAc)_2$ (1.5 equiv), MeCN/CH_2Cl_2 (1:1), N_2, 20 °C, 24 h. [b] Other products formed but not identified.

Table 2. Yields of products obtained from Fe^{2+}-mediated decomposition of artemisinins **5** and **6**.



24: R = H; **29**: R = *p*-fluorophenyl; **25**: R = H; **30**: R = *p*-fluorophenyl; **26**: R = H; **31**: R = *p*-fluorophenyl; **27**; **28**

Starting Artemisinin R Group (Figure 2)	Method[a]	Artemisinin Remaining and Products [%]	Reaction Half-Life [h]
5: 10-deoxoartemisinin	a	**5**: 3, **24**: 42, **25**: 42, **26**: 4, **27**: 2	6.0
R = H	b	**5**: 0, **24**: 54, **25**: 40, **26**: 6	3.0
	c	**5**: 3, **24**: 13, **26**: 63, **27**: 9, **28**: 9	
	cl	**5**: 0, **26**: 79, **28**: 8	
	d	**5**: 1, **24**: 24, **26**: 20, **27**: < 2	
	e	**5**: 17, **26**: 67	
6	a	**6**: 96, **29**: < 1, **30**: 2, **31**: 2	> 24
R = –*p*-fluorophenyl	b	**6**: 94, **29**: < 1, **30**: 4	> 24
	c[b]	**6**: 17, **29**: 14, **31**: 17	

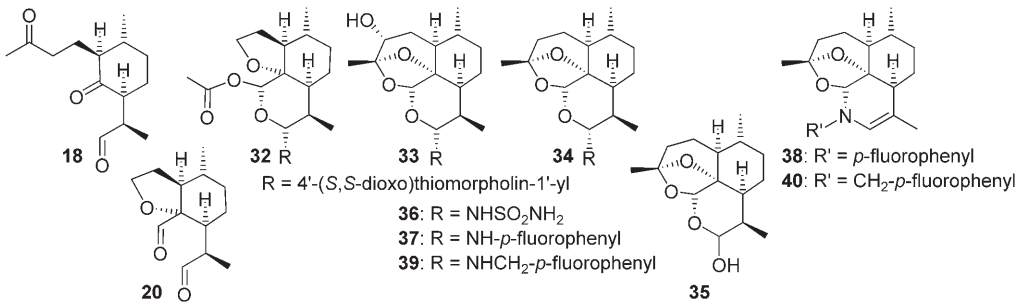
[a] Method a) $FeSO_4$ (0.3 equiv), $MeCN/H_2O$ (1:1), N_2, 20 °C, 24 h; method b) as for a + ascorbic acid (AA, 1.0 equiv); method c) $FeBr_2$ (0.5 equiv), THF, N_2, 20 °C, 45 min; method cl) from Ref. [53]: $FeBr_2$ (1.94 equiv), THF, N_2, 20 °C, 15 min; method d) $Fe(OAc)_2$ (1.5 equiv), $MeCN/CH_2Cl_2$ (1:1), N_2, 20 °C, 24 h (cf. Ref. [51]); method e) **5** (neat solid), 100 °C, N_2, 18 h. [b] Other products formed but not characterized.

40 represent a new structural type. Their relationship with the 2-deoxyglycal **19** obtained from DHA (Table 1) is brought out below.

b. Radical interception with 4-oxo-TEMPO

Attempts to intercept radicals by treatment of 1:2 mixtures of DHA **2** or the 10-aminoartemisinin **11** and 4-oxo-TEMPO with ferrous acetate in 1:1 acetonitrile/dichloromethane during 24 h at 20 °C according to the method used to intercept radicals from the trioxolane **13**[51] gave only variable amounts of the products arising from the artemisinins alone (cf. Tables 1 and 3). Treatment of a 1:2 mixture of

Table 3. Yields of products obtained from Fe^{2+}-mediated decomposition of 10-aminoartemisinins **7**–**12**.

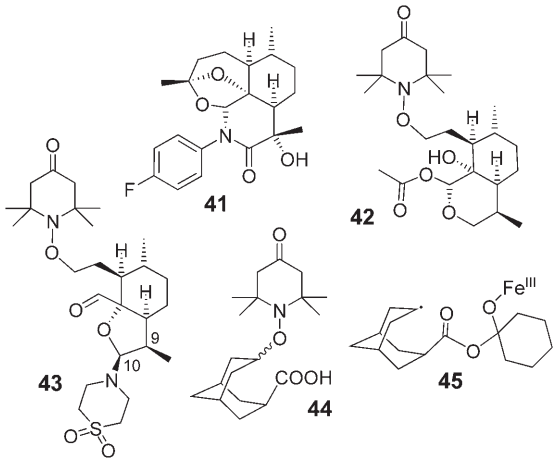


18; **20**; **32** R; **33** R; **34** R; R = 4'-(*S*,*S*-dioxo)thiomorpholin-1'-yl; **36**: R = $NHSO_2NH_2$; **37**: R = NH-*p*-fluorophenyl; **39**: R = $NHCH_2$-*p*-fluorophenyl; **35**; **38**: R' = *p*-fluorophenyl; **40**: R' = CH_2-*p*-fluorophenyl

Starting Artemisinin R Group (Figure 2)	Method[a]	Artemisinin Remaining and Products [%]	Reaction Half-Life [h]
7: artemisone	a	**7**: 14, **18**: 5, **20**: 16, **33**: 28	9.6
R = 4′-(*S*,*S*)-dioxo-thiomorpholin-1′-yl	b	**7**: 3, **18**: 6, **20**: 14, **33**: 20	3.1
	c[b]	**7**: 0, **18**: 22, **20**: 29, **34**: 9	
	d[b]	**7**: 0, **18**: 3, **20**: 11, **32**: 12, **33**: 8	
8	a	**8**: 96, **18**: ~1, **20**: 3	> 24
R = 4′-benzylpiperazin-1′-yl	b	**8**: 29, **18**: 12, **20**: 27	
	c	**8**: 0, **18**: 26, **20**: 26, **35**: 24	
9	a	**9**: 97, **18**: ~1, **20**: 2	> 24
R = 4′-(1″,3″-pyrimid-2″-yl)piperazin-1′-yl	b	**9**: 68, **18**: 2.5, **20**: 12	
10	a[b]	**10**: 48, **18**: 2, **36**: 8	
R = –$NHSO_2NH_2$	c[b]	**10**: 0, **18**: 32, $H_2NSO_2NH_2$: 63	
11	a	**11**: 61, **37**: 27	
R = –NH-*p*-fluorophenyl	c[b]	**11**: 0, **18**: 24, **38**: 12	
	d[b]	**11**: 61, **37**: 2, **38**: 1	
12	c[b]	**12**: 0, **18**: 5, **39**: 5, **40**: 24	
R = –$NHCH_2$-*p*-fluorophenyl			

[a] Method a) $FeSO_4$ (0.3 equiv), $MeCN/H_2O$ (1:1), N_2, 20 °C, 24 h; method b) as for a + ascorbic acid (AA, 1.0 equiv); method c) $FeBr_2$ (0.5 equiv), THF, N_2, 20 °C, 45 min; method d) $Fe(OAc)_2$ (1.5 equiv), $MeCN/CH_2Cl_2$ (1:1), N_2, 20 °C, 24 h. [b] Other products formed (≤ 5 %) but not characterized.

10-deoxoartemisinin **5** and 4-oxo-TEMPO with ferrous acetate (1.5 equiv) gave the radical-trapped product **42** (3%), and the products of Table 2. Artemisone provided the product **43** in 10% yield. Treatment with ferrous acetate (3 equiv) of a 1:1:2 mixture of **5**, **13**, and 4-oxo-TEMPO provided **42** (3%) and **44** (73%) arising by entrapment of the seco-radical **45** from **13**.[51] Thus, the inability of 4-oxo-TEMPO to intercept significant amounts of C radicals from the artemisinins is not due to an experimental artifact. Identification of **42** follows through a consideration of its spectroscopic data and general similarity with that of **24** (Table 2). Compound **43** is characterized through its spectroscopic data (Experimental Section). In particular, the ^{1}H NMR spectrum indicates a cis-coupling of 2.9 Hz between H-10 and H-9. The genesis of this compound is discussed below.



c. Effect of desferrioxamine on biological properties

The effect of iron on PfATP6 inhibition was evaluated with desferrioxamine B (DFO) (Figure 3). Although DFO attenuates inhibition of PfATP6 by artemisinin **1** (500 nM),[45] ascorbic acid (AA) countered the effect of DFO, although AA alone had no effect on inhibition. Inhibition of PfATP6 by artesunate **3** (250 nM) and 10-deoxoartemisin **5** (100 nM), both rapidly decomposed by aqueous Fe^{2+} (Tables 1 and 2), was attenuated by DFO. In contrast, DFO did not significantly suppress inhibition by the *p*-fluorophenyl derivative **6** (250 nM), artemisone **7** (5 nM), and the pyrimidylpiperazine derivative **9** (100 nM) (Figure 3). Significantly, compounds **6** and **9** are inert to aqueous Fe^{2+}.

The effect of DFO on antimalarial activity (Figure 4) displays a

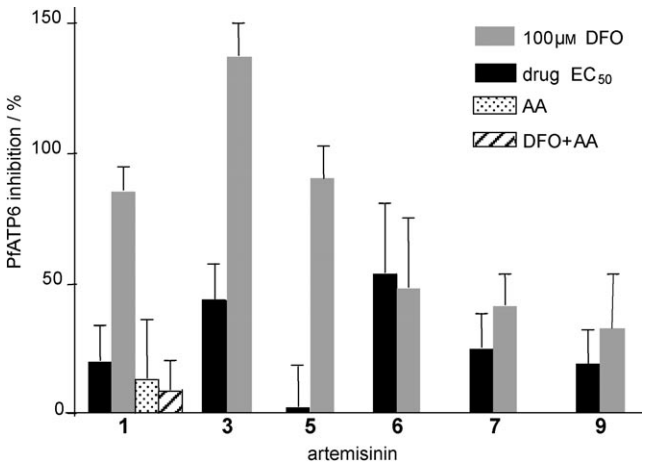


Figure 3. Inhibition of PfATP6 by artemisinins (drug concentrations between EC_{50} and EC_{90}, black columns) and effect of desferrioxamine (DFO, 100 μM, gray columns) (data for artemisinin **1**, 500 nM, from Ref. [45]). Effect of ascorbic acid (AA, 100 μM, stippled column) on inhibition of PfATP6 by **1**, and effect of a mixture of AA and DFO (each 100 μM, diagonals) in restoring inhibition of PfATP6 by **1**; inhibition of PfATP6 by artesunate **3** (500 nM) and 10-deoxoartemisinin **5** (100 nM) and attenuation by DFO (100 μM, $p < 0.001$); inhibition of PfATP6 by 4-fluorophenylartemisinin **6** (250 nM, $p = 0.8$) and artemisone **7** (5 nM, $p = 0.26$) and relative lack of effect of DFO ($p > 0.05$). DFO has little effect on inhibition of PfATP6 by the pyrimidylpiperazine derivative **9** (100 nM, $p = 0.6$). Data (mean ± SEM) are from 6–10 experiments, except for **6** ($n = 3$); effects of DFO are assessed with Students (unpaired) *t* test.

provocative parallel with that on PfATP6 inhibition. DFO antagonized the antimalarial activities of the aqueous Fe^{2+}-susceptible artesunate **3** and 10-deoxoartemisinin **5**, although it had no observable effect on the aqueous Fe^{2+}-resistant *p*-fluorophenyl derivative **6** or on artemisone **7**.

The inhibition constant (K_i) for artesunate **3** against PfATP6 of 59.2 ± 7.3 nM and the IC_{50} value of 6 ± 3 nM against *P. falciparum* (3D7) represents an approximate 10-fold difference in inhibitory potencies. Artemisone **7**, with $K_i = 1.68 \pm 0.63$ nM and an IC_{50} value of 0.6 ± 0.22 nM, demonstrates the smallest

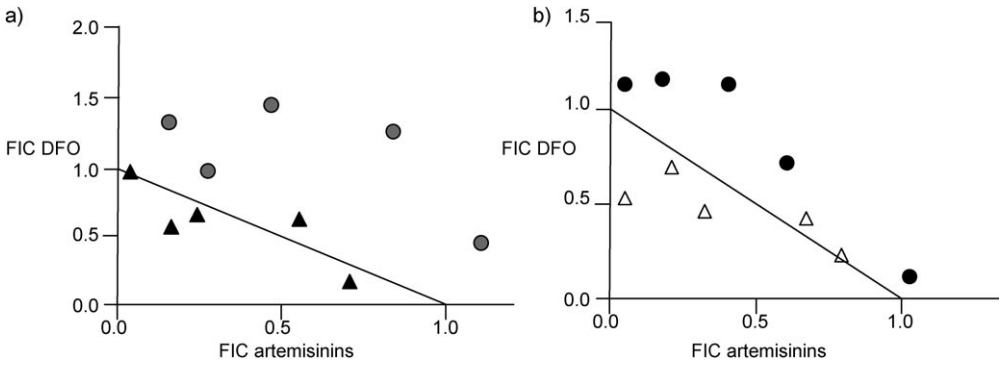


Figure 4. Isobologram analyses of the effect of desferrioxamine B (DFO) on antimalarial activities of artemisinins against *P. falciparum* 3D7**:** a) DFO and 10-deoxoartemisinin **5** (●), DFO and artemisone **7** (▲); b) DFO and artesunate **3** (●), and DFO and 4-fluorophenylartemisinin **6** (△). The solid lines represent isoboles at which the drugs act independently and additively. Data points above these lines indicate antagonism. After determination of IC_{50} values (within 48 h of adding antimalarials/inhibitors), fixed ratios of DFO to that of the artemisinin were used; these ratios are: 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, 0:10. The middle concentration of the inhibitors (5:5) approximates the IC_{50} value of each inhibitor. The molar ratios of each inhibitor were calculated and the IC_{50} values of the mixtures presented in relation to the IC_{50} of the individual inhibitors.

discrepancy (threefold).[46] However, the K_i value of 277 ± 39 nM for 4-fluorophenylartemisinin **6** against PfATP6 is almost 200-fold higher than its corresponding IC_{50} value (1.44 ± 0.28 nM, 3D7). Compound **6** is highly lipophilic (log*P* 5.59; cf. artemisone **7**, log*P* 2.08) and is insoluble in water (< 1 mg L^{-1}; cf. artemisone **7**, 89 mg L^{-1}).[49] Therefore, competitive partitioning of **6** into the membrane lipids constituting the expression system used for the oocyte SERCA assays[58] will occur, decreasing the concentration of **6** available for binding. In contrast, selective concentration of artemisinins takes place in infected erythrocytes[59] wherein the more lipophilic artemisinins express very good activities in vivo against the malaria parasite.[48]

Antimalarial activity of some of the artemisinins examined herein are given in Table 4. According to this and other in vitro and in vivo data,[48] artemisone **7** ranks among the most active of all peroxidic antimalarials.[50] Trioxolane **13** possesses an IC_{50} value of 4.5 nM against the K1 strain.[50,51]

Table 4. In vitro inhibition data for artemisinins against W2 (chloroquine-resistant) and D10 (chloroquine-sensitive) strains of *P. falciparum*.

Compd[a]	IC_{50} [nM]		Compd[a]	IC_{50} [nM]	
	W2	D10		W2	D10
1	4.82 ± 0.48	7.33 ± 2.21	**9**	1.53 ± 0.51	2.04 ± 0.49
5	1.71 ± 0.10	3.39 ± 0.71	**10**	3.03 ± 0.98	7.89 ± 2.94
7	0.60 ± 0.25	0.72 ± 0.24	**11**	0.82 ± 0.39	2.07 ± 1.73
8	0.66 ± 0.27	1.17 ± 0.86			

[a] See Figure 2 for structures.

Discussion

a. Products of Fe^{2+}-mediated decomposition

Before focusing on the wider implications of these results, we subdivide the products into type **a** arising via Fenton-like cleavage of the peroxide by Fe^{2+} and intramolecular closure of the resulting C radicals with extrusion of Fe^{2+} (cf. Figure 1), type **b** arising via formal peroxide cleavage and loss of formic acid,[55] and type **c** arising via reduction (Figure 5). Formation of products **aiii** is favored by aqueous conditions, and products **b** and **c** by anhydrous conditions, becoming predominant for DHA **2** (Table 1) and for **5** and **6.** Product types **aii** and **bi** arise from artemisinins bearing a leaving group at C10. Products **a** are formed in significant amounts under aqueous conditions from artemisinins **1**–**3**, **5**, and **7**. The other aminoartemisinins tend to be less amenable to decomposition under aqueous conditions. In general, ferrous acetate under anhydrous conditions was relatively ineffective, and of the artemisinins examined, only **5** and **7** provided appreciable amounts of products. DHA **2** and the anilino artemisinin **11** are essentially inert.

The mode of formation of decomposition products has been exhaustively discussed,[9,10,17,18,54] and we confine our attention here to the products arising from the aminoartemisinins **7**–**12** (Figure 6). When the decomposition provides intermediates **46** and **48** with a leaving group attached either directly to C10 as in **46** (formate), or via an electronically connected framework as in **48** (acetate), the amino group induces fragmentation to give the iminium ions **47** and **49**, which will undergo hydrolysis to the carbonyl compounds on workup of the reaction mixture. In the case of the sulfamido artemisinin **10**, the amine product of hydrolysis, the insoluble sulfamide ($H_2NSO_2NH_2$) is recovered. The formation of the radical-trapped product **43** diverts the seco-C4 radical away from the furan-forming pathway leading to **48**. Entrapment with 4-oxo-TEMPO is followed by extrusion of acetate and Fe^{3+}, and intramolecular cyclization of the pendant hydroxyl with the iminium group in intermediate **50** to give **43**. This has the interesting consequence that the overall reaction will no longer be catalytic in Fe^{2+}. Formation of the trapped product **42** from 10-deoxoartemisinin, which contains no electrofugal group, is consistent with the proposal of Figure 6. The pathway leading via the C4 radical and epoxide **51** is disfavored under nonaqueous conditions; the inference is that the incipient O2 radical prefers to undergo cleavage to provide the tricarbonyl compound **46**, a premise which is consistent with a non-concerted cleavage of peroxides to provide carbonyl compounds.[54,55] The alternative pathway to the carbonyl compounds involves an Fe^{2+}- (or Fe^{3+}-) mediated heterolytic decomposition of the peroxide, formally a [2+2+2] cycloreversion, for which the thermal counterpart is demonstrated in the thermal decomposition of 10-deoxoartemisinin **5** (Table 3).

The formation of the products **c** may proceed via reduction of a hydroperoxide intermediate arising via ring opening[21] and re-closure of the resulting alcohol.[60] Ring opening of DHA to the hydroperoxide **52** (X = H, Y = O, Figure 7) is implicated in the facile formation of a rearranged peroxyhemiacetal from DHA.[55] In the present case, ring opening of DHA and the artemisinins **11** and **12** may be driven by protonation of the peroxide or by complexation with Fe^{2+}. The hydroperoxides **52** on

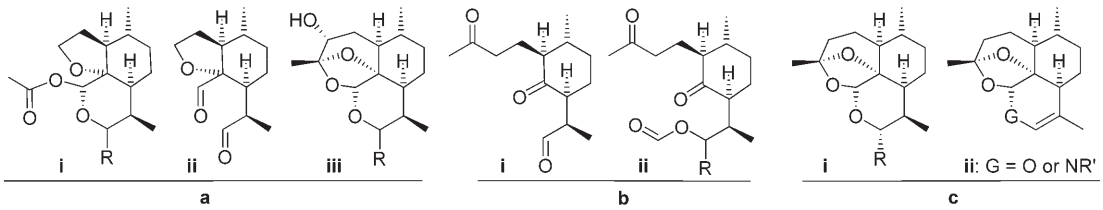


Figure 5. Product types obtained from Fe^{2+}-catalysed conversion of artemisinins: **a**, 'radical marker' products (cf. Figure 1); **b**, cleavage products; **c**, reduction products.

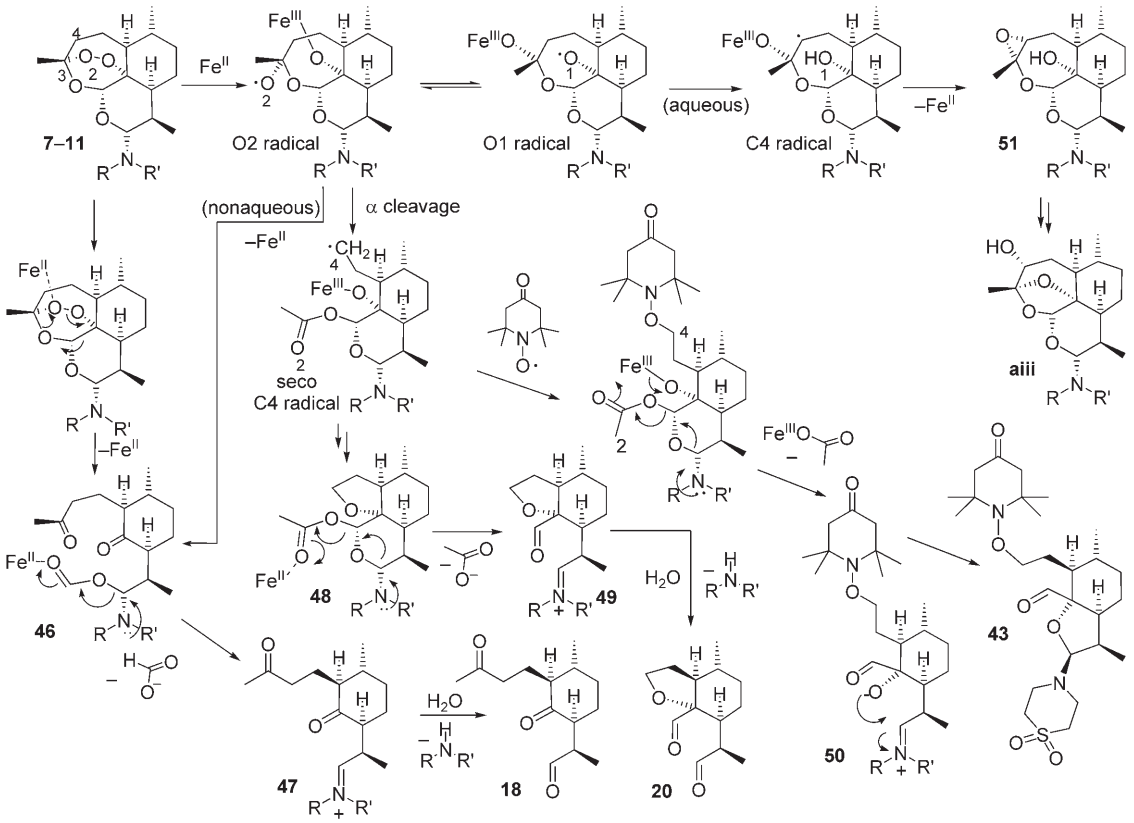


Figure 6. Pathways proposed for the formation of products from aminoartemisinins **5**–**7** and radical-trapped product **43** from artemisone **7**.

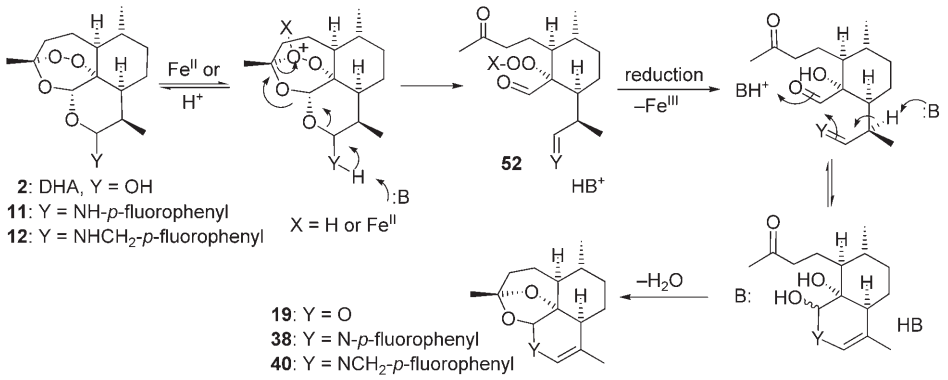


Figure 7. Proposal for the formation of dehydroglycal **19** from DHA **7** and enamines **38** and **40** from DHA and primary aminoartemisinin derivatives **11** and **12**.

reduction give the corresponding alcohol, which close to the 2-deoxyartemisinins **ci**. Alternatively, closure of the alcohols arising via reduction of **52** via the corresponding enol or enamine intermediates provide the enol and enamine products **19**, **38**, and **40**.

b. Ramifications for antimalarial mechanism of action

i. Fe^{2+}-mediated decomposition and C radicals

Artemisone **7** does not provide significantly greater amounts of radical marker products **a** (Figure 6) than does artemisinin **1**, even though inhibition of PfATP6 and antimalarial activity are vastly superior. In general, the aminoartemisinins are more refractory to reduction by aqueous Fe^{2+} than other artemisinins. The propensity of artemisinins to undergo decomposition is not related to aqueous solubility. Although artemisinins **5** and **11** have aqueous solubilities of 294 and 295 mg L^{-1} at pH 7.2, the former is almost completely decomposed by aqueous Fe^{2+}, and the latter is substantially more inert. Artemisone **7**, with an aqueous solubility of 89 mg L^{-1}, is more rapidly decomposed than artemisinin **1**, with a solubility of 63 mg L^{-1}. The benzylpiperazine derivative **8**, with an aqueous solubility of 8 mg L^{-1} is essentially inert to aqueous Fe^{2+}, but ascorbic acid enhances the decomposition.

It is clear that under either aqueous or anhydrous conditions, Fe^{2+} encumbered with oxygen ligands is less active as a decomposition catalyst than are ferrous halides.[9,10,17,43,61] The rate-determining step is displacement of the ligand, more facile for halide, by peroxide, to form a complex in which inner-sphere electron transfer from Fe^{2+} to the peroxide initiates the Fenton-like cleavage.[62] The amino groups of the 10-

aminoartemisinins will associate with Fe^{2+} to form complexes that may either slow the rate of decomposition, or for steric reasons hinder formation of the complex between Fe^{2+} and the peroxide.[48] The enhanced decomposition of the benzylpiperazine and pyrimidinylpiperazine derivatives **8** and **9** induced by ascorbic acid may be due to competitive generation of an ascorbic acid–Fe^{2+} complex[63] that reduces the peroxide. Be that as it may, on the basis of the C radical hypothesis, the acceleration of the aqueous Fe^{2+} decompositions by ascorbic acid is out of key with the antagonism of antimalarial activities reported for this reagent,[30,52] given that the (faster) reactions leading to the radical marker products **a** imply an enhanced flux of 'bioactive' C radical intermediates.

The radical center in the seco-radical **45** from trioxolane **13**, embedded in a rigid hydrocarbon skeleton, is amenable to efficient intermolecular trapping to provide **44**. In contrast, the formation in poor yields of radical-trapped products **42** and **43** from artemisinins **5** and **7** indicates that internal quenching of the structurally relatively complex and flexible seco-C4 radical intermediates (cf. Figures 1 and 6) via extrusion of Fe^{2+} to provide products **ai**–**ii** is facile. In addition, generation of the artemisinin C radicals competes with Fe^{2+}-mediated cleavage of the peroxide to provide carbonyl compounds **b** (cf. Figure 6), a decomposition pathway which is not available to trioxolane **13**. That is, the intercession of this manifold of intramolecular decomposition pathways of the artemisinins triggered by Fe^{2+} implies that the concentration of the C radical intermediates available for an intermolecular reaction with a crucial biomolecule will be low indeed, even in the situation where the artemisinin may be bound in a protein cleft, such as the TG-sensitive cleft of PfATP6. This factor aside, we have already pointed out[13] that those elegantly constructed analogues of artemisinins lacking C3 and/or C4,[64] and a rearrangement product of DHA, which is incapable of reverting to DHA,[42,55] are active antimalarials, even though formation of C radicals are not apparent for these substrates.

In light of the foregoing, the recent idea[65] that products of type **ai** may be taken as markers of the biological importance of the seco-C4 radical precursors (cf. Figures 1 and 6) is noteworthy. The formation of these compounds in limited yields under aqueous conditions, and the inability to effectively trap the C radical precursor with the optimal, although non-biological reagent—the stable radical 4-oxo-TEMPO—implies that in the intracellular environment, the likelihood of the C radical precursor damaging a vital biomolecule by whatever mechanism must be small. Finally, the successful trapping of C radical **45** by the stable O-centered radical cannot be used to support the assumption that such C radicals are the actual cytotoxic intermediates in biological systems. C Radicals associated with a saturated flexible framework are unsuited per se for alkylation of biomolecules.[13] Suffice it to say that they are reduced by thiols, as has been recorded in the artemisinin area,[10,18] and react at a diffusion-controlled rate with oxygen. An inference that such radicals may react with oxygen has been made.[61] C Radicals are generated by treatment of oxaziridines with Fe^{2+}, yet the compounds are either inactive or feebly active as antimalarials.[66]

ii. Interpretation of the effects of Fe^{2+}, ascorbic acid, and DFO on biological properties

The observations recorded herein, namely that ascorbate accelerates the decomposition and that DFO antagonizes the PfATP6 binding and antimalarial activities of the aqueous Fe^{2+}-susceptible, but not that of the aqueous Fe^{2+}-insusceptible artemisinins, are not easily reconciled with current dogma on the mechanism of action of artemisinin antimalarials. Furthermore, if the C radicals are not viable intermediates, then, if it is accepted that an interaction of the artemisinin with PfATP6 is a prerequisite to parasite death, a different kind of reactive intermediate from the artemisinin which irreversibly modifies the transporter or destroys a crucial endogenous substrate, such as a cofactor associated with the transporter, must be involved.

The Fe^{2+} available for activation of the artemisinins within the parasitized erythrocyte is presumed to be associated with the extra- and intracellular labile iron pools containing redox-active iron complexes, which are subject to scavenging by chelators, and which are responsible for mediating generation of reactive oxygen species (ROS) and modulating oxidative stress.[67,68] Thus, those artemisinins **1**, **3**, and **5** susceptible to aqueous Fe^{2+}-mediated decomposition may suffer competitive degradation in the aqueous extracellular medium or intracellular aqueous cytosolic compartments by the labile Fe^{2+}. The antagonism of antimalarial activity by ascorbic acid is then ascribed to the greater concentrations of Fe^{2+} generated by ascorbic acid enhancing this degradation. DFO both attenuates PfATP6 inhibition by the aqueous Fe^{2+}-susceptible artemisinins and antagonizes their antimalarial activities, but has relatively little effect on the aqueous Fe^{2+}-resistant artemisinins **6** and **9**. This observation is not easily explained. However, DFO, via the Fe^{3+} complex ferrioxamine (FO), likely sequesters and concentrates iron stores, probably in aqueous compartments in the cytosol.[35] Exposure to an endogenous reductant such as glutathione or ascorbate[35] may produce locally high concentrations of Fe^{2+} that competitively destroy the susceptible artemisinins, but have little effect on the aqueous Fe^{2+}-inert artemisinins **6** and **9**. Ascorbic acid neutralizes the attenuating effect of DFO on inhibition of PfATP6 by **1** (Figure 3) because Fe^{3+} is no longer available for sequestration by DFO. Alternatively, artemisinins may undergo ring opening to provide free hydroperoxides (cf. **52**, Figure 7) in the intracellular environment.[17,21,42,60] Such ring opening of artemisinin and DHA, the principle metabolite of the clinically used artemisinins artesunate **3** and artemether **4**, occurs under mild conditions in the laboratory,[55,60] although this has yet to be demonstrated for artemisinins bearing other groups at C10. In any event, ascorbate, DFO, or endogenous reductant in the aqueous intracellular environment will destroy the hydroperoxides derived from the aqueous soluble artemisinins, decreasing the amount of drug available for a parasiticidal effect.

Irrespective of the exact mode of attrition, 10-deoxoartemisinin **5**, with an aqueous solubility of 294 mg L^{-1}, is highly susceptible to decomposition by aqueous Fe^{2+}, and DFO attenuates its inhibition of PfATP6 and antagonizes antimalarial activi-

ty. Artemisone **7**, with its good physicochemical properties, is efficiently transferred to the target,[46] and the effect of DFO is less pronounced. The fluorophenyl derivative **6** and aminoartemisinins such as **8** are not readily decomposed by aqueous Fe^{2+}; that is, they cannot suffer competing degradation en route to the target. Thus, DFO does not overtly influence binding to PfATP6 or antimalarial activities of these compounds. It is of interest to note that **6** and other lipophilic aminoartemisinins generally display greater antimalarial activities (and higher toxicities) than do more polar derivatives.[4,43,48] These compounds will be less prone to enter the aqueous cellular compartments and will localize and migrate within lipophilic structures such as organelle membranes; that is, they are protected from adventitious decomposition. In common with the action of lipophilic hydroperoxides, biological activities will not be greatly impaired by the presence of iron chelators, which are unlikely to access iron in a lipophilic environment.[69]

The antimalarial activities and their modulation by DFO, and the effect of artemisinins on the thapsigargin (TG)-sensitive SERCA ATP6 target have an intriguing parallel with the biological properties of structurally simple peroxides that cannot readily generate C radicals. This parallel in antimalarial activities has been brought out in a classical review of oxidant drugs and malaria.[70] *tert*-Butyl hydroperoxide (TBH) induces rapid reduction in parasitemia in mice infected with *P. vinckei*, whereas it has no effect on uninfected mice,[71] and is parasiticidal in vitro towards *P. falciparum*.[72] In relation to its effect on Ca^{2+} transporters, TBH increases intracellular Ca^{2+} levels in PC12 pheochromocytoma cells. Pretreatment with TG abolishes the effect of TBH, whereas pretreatment with TBH abrogates the TG-induced increase in Ca^{2+}.[73] Hydrogen peroxide at micromolar concentrations is parasiticidal in vitro and in vivo against *P. yoelii* and in vitro against *P. falciparum*.[70] The effect of H_2O_2 in modulating intracellular Ca^{2+} levels is well known.[74] As one pertinent example, it increases cytosolic and mitochondrial Ca^{2+} concentrations in pancreatic cells; pretreatment with TG inhibits the H_2O_2-induced changes. Thus, H_2O_2, like TBH, exerts an effect on TG-sensitive Ca^{2+} ATP transporters in mammalian cells.[75] Artemisinins at micromolar levels induce apoptosis in mammalian cells by modulating cytosolic Ca^{2+} levels, although the direct cause has yet to be pinpointed.[76] To summarize, the hallmark of oxidant stress as elicited by all peroxidic compounds is overt modulation of intracellular Ca^{2+} flux[77] leading to apoptosis, such as may be effected by inhibition of the TG-sensitive ATP transporter PfATP6 in the parasite.

Thus, a proper appreciation of the manner in which artemisinins, and apparently simple hydroperoxides, interact with the transporter becomes crucially important. In this respect, it is unclear how structurally diverse peroxides such as 10-deoxoartemisinin **5**, the pyrimidinylpiperazine derivative **9**, trioxolane **13** and its close relatives,[50,78] and a steroidal tetroxane,[79] which possess antimalarial activities in vitro ranging from 1.5–11.4 nM may bind into the TG cleft to inhibit parasite PfATP6. For example, the trioxolane, OZ OZ277 (RBx6660) is two orders of magnitude less effective in inhibiting PfATP6 (K_i = 7700 nM) than is artemisinin (K_i = 79 nM), an interaction which is also apparently sensitive to DFO.[78] Compounding this quandary are the observations that replacement of residues lining the TG binding cleft in PfATP6 by orthologous residues affects the inhibition by artemisinin and artemisone, and that mammalian SERCAs are at least an order of magnitude less sensitive to these artemisinins.[46] Although artemisinin reduces mitochondrial inner membrane potential in a yeast model through iron-dependent generation of ROS,[80] it is unlikely our Fe^{2+}-resistant artemisinins will elicit such an effect. The non-neurotoxic artemisone **7**, unlike artemether or DHA, has no effect on mitochondrial membrane potential, on ROS levels, or on inhibition of the respiratory chain in neuronal cell lines.[81] Therefore, the proposal that artemisinins exert their antimalarial activity by interfering with mitochondrial function[80] is premature.

Conclusions

The PfATP6 inhibition and antimalarial mechanism is independent of reductive cleavage of the peroxide by Fe^{2+} leading to C radicals.[12] The potent antimalarial activities of our aminoartemisinins are inconsistent with their demonstrably inefficient conversion into C radicals, which further, because of facile intramolecular quenching pathways, cannot be fruitfully intercepted. In contrast, the trioxolane **13** provides a good yield of a radical-trapped product, yet possesses lower antimalarial activity (higher IC_{50} value). As an explanation for the antimalarial activity of artemisinins, or indeed of the biological activities of artemisinins at large, the C radical hypothesis is not feasible.

Artemisinins, trioxolane, and tetroxane analogues, as peroxide carriers, are capable of drastically influencing intracellular events, as has been established for hydrogen peroxide and alkyl hydroperoxides. As artemisinins have the peroxide encapsulated within a robust tricyclic scaffold, they are less prone to undergo adventitious decomposition such as reduction or Haber–Weiss chemistry characteristic of unprotected hydroperoxides. If binding to PfATP6 is a crucial step in the pathway to destruction of the malarial parasite, it needs to be established if this is associated with an irreversible chemical change in the cleft, or destruction of a crucial endogenous substrate such as a cofactor, which occurs because of the binding, and which has the consequence of inhibiting overall function of the transporter. The precise role of the peroxide remains to be defined. If an open hydroperoxide is involved, it can act as an electrophilic oxygenating agent, or provide hydroxyl or alkoxyl radicals now capable of reacting with biological substrates.[17,21,43,60] It should be noted that the intercession of the Haber–Weiss chemistry leading to O-centered radicals is not necessarily dependent on the presence of metal ions.[82] Alternatively, the hydroperoxide or the intact peroxide may act as an electron acceptor (oxidant), for example, in disrupting a catalytic cycle involving the cofactor.[83] The important difference here is that it is the intrinsic reactivity of the peroxide which confers antimalarial activity, not the erratically derived and fickle C radicals.

The intraerythrocytic malaria parasite is in an environment which is oxidatively stressed.[70,83,84] Thus, enhanced levels of methemoglobin, of lipid peroxidation, increased intracellular flux of hydrogen peroxide and ROS, and other effects render

intraerythrocytic parasites peculiarly susceptible to modulation of oxidative stress. Substantially lower quantities of artemisinins—on the order of nanomolar concentrations—are required to kill the intraerythrocytic parasite, whereas oxidatively unstressed mammalian cells require larger amounts of artemisinins—in the micromolar range—to elicit cytotoxic effects.[65,85] Fe^{2+} profoundly modulates oxidative stress in its own right.[67,86] Thus, the enhancement of cytotoxicities of artemisinins in mammalian cells[87] by addition of Fe^{2+} is unsurprising.

At the proof stage of this manuscript, a publication appeared presenting details of an examination of the effects of DFO and the more lipophilic chelator deferiprone on the antimalarial activities of a series of peroxidic antimalarials.[88] The chelators were shown to antagonize the antimalarial activities of selected peroxides, including artemisone. It is important to note that the "antagonism" does not mean destruction of antimalarial activity. The drugs are still substantially antimalarial active in the presence of the chelators; therefore, extrapolation of the authors' interpretation of the role of the chelators implies that there must be a "non-iron" pathway of activation. The possibility of the chelators reacting with the artemisinins or putative peroxide intermediates aside, if one function of the chelators is to remove labile iron, this will alleviate oxidative stress, and more of the artemisinin is now required to kill the parasite, as explained in the preceding paragraph. Thus the data in reference [88], including the use of normal nonproliferating human PBMC as a control, do not imply at all that peroxidic antimalarials have to react chemically with iron to exert their antimalarial effect.

Experimental Section

Artemisinin **1** and DHA **2** were purchased from the Kunming Pharmaceutical Corporation (PR China) or from Haphacen, Hanoi College of Pharmacy (Vietnam) and used without further purification. Artesunate **3** was prepared according to the Chinese procedure.[3] The other derivatives were prepared as previously described.[43,48] 4-Oxo-TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, free radical) was used as received from Sigma–Aldrich.

All reactions were carried out under an atmosphere of N_2. The following solvents were dried prior to use: ethyl acetate from $MgSO_4$, hexanes ($CaCl_2$), CH_2Cl_2 (CaH), triethylamine (CaH and stored over KOH pellets), and tetrahydrofuran (sodium in benzophenone). TLC was performed with Merck Kieselgel 60 F_{254} plates and visualized with UV light (254 nm) and/or heating after treatment with 5% ammonium molybdate in 10% concentrated H_2SO_4. Column chromatography was performed with Merck silica gel 60 (0.04–0.063 mm). ^1H and ^{13}C NMR spectra were obtained as solutions in $CDCl_3$ on a Varian Mercury spectrometer operating at 300 and 75 MHz, respectively. Melting points were carried out on a Leica Hot Stage DME E compound Microscope and are corrected. MS data were obtained on a Finnigan TSQ 7000 mass spectrometer (CI+, methane), on an API QSTAR high-performance triple-quadrupole time-of-flight (ToF) mass spectrometer with electrospray ionization, and on a Waters Micromass GCT premier ToF high-resolution mass spectrometer (CI+, methane). IR spectra were recorded either on a PerkinElmer PC 16 or a PerkinElmer Spectrum One spectrometer. Optical rotations were performed on a PerkinElmer model 241 spectrometer. Elemental analyses were obtained from MEDAC Ltd., Surrey (UK).

1) In vitro decomposition of artemisinin derivatives with Fe^{2+} salts

a) **$FeSO_4$**: A solution of $FeSO_4 \cdot 7H_2O$ (33.2 mg, 0.3 equiv) in deionized H_2O (4 mL) was added dropwise to a stirred solution of the artemisinin derivative (0.4 mmol) in CH_3CN (4 mL) under N_2 at room temperature, and the mixture was stirred for 24 h. It was then extracted with CH_2Cl_2 (3×20 mL) and the combined organic layer was washed with deionized H_2O (20 mL). The organic layer was separated and dried ($MgSO_4$). After filtration and concentration of filtrate under reduced pressure, the amounts of unreacted starting material and the products in the crude product mixture were estimated by ^1H NMR spectroscopy of the product mixtures as described below. The components were also separated by chromatography with ethyl acetate/hexanes as eluent to give the unreacted artemisinin derivative and the decomposition products. Products were weighed and identified either by comparison with reported data, or if new, by spectroscopic methods as described below. Yields as given below refer to the amounts of starting material and products isolated from the reaction mixture.

b) **$FeSO_4$–ascorbic acid**: According to procedure a), a solution of $FeSO_4 \cdot 7H_2O$ (33.2 mg, 0.3 equiv) and ascorbic acid (AA, 70.4 mg, 0.4 mmol, 1.0 equiv) in deionized H_2O (4 mL) was added dropwise to a stirred solution of the artemisinin derivative (0.4 mmol) in CH_3CN (4 mL). Products were either isolated or estimated by ^1H NMR spectroscopy as described below.

a) and b) **$FeSO_4$ and $FeSO_4$–ascorbic acid and reaction half-lives**: *Set I*: A solution of $FeSO_4 \cdot 7H_2O$ (74.7 mg, 0.3 equiv) in deionized H_2O (9 mL) was added dropwise to a stirred solution of the artemisinin derivative (0.9 mmol) in CH_3CN (9 mL) under N_2 at room temperature. *Set II*: A solution of $FeSO_4 \cdot 7H_2O$ (49.8 mg, 0.3 equiv) in deionized H_2O (6 mL) was added dropwise to a stirred solution of the artemisinin derivative (0.6 mmol) in CH_3CN (6 mL) under N_2 at room temperature. Aliquots (3.4 mL) of the reaction mixture were withdrawn (at 2, 3, 4, 6, and 10 h for Set I, and at 14, 18, and 24 h for Set II), followed by extraction with CH_2Cl_2 (3×10 mL), and the organic layer in each case was washed with deionized H_2O (10 mL). The organic layer was separated and dried ($MgSO_4$). After filtration and concentration of the filtrate under reduced pressure, the residue was taken into $CDCl_3$ containing 1,3,5-trimethoxybenzene (~5.0 mg) as an internal standard. ^1H NMR measurements were conducted by comparing the intensity of the aromatic signal in the standard at 6.08 ppm against the singlet due to H-12 in each of the artemisinins (artemisinin **1** 5.86, artesunate **3** 5.44, 4-fluorophenyl **6** 5.55, artemisone **7** 5.27, benzylpiperazine derivative **8** 5.35, pyrimidinylpiperazine derivative **9** 5.27, sulfamide **10** 5.38, 4′-fluorophenylamino **11** 5.41, 4′-fluorobenzylamino **12** 5.27 ppm). As an independent check, starting material was also isolated in a pure state by chromatography, weighed, and constitution verified by spectroscopic analysis. In the case of the 10-deoxo compound **5**, the signal due to H-12 (5.20 ppm) overlaps the signal of H-12 in the decomposition product **25** in the ^1H NMR spectra of the product mixtures. Therefore, the rate of decomposition of 10-deoxoartemisinin was followed by monitoring changes in peak intensities of the signal due to the methyl group at C3 (1.48 ppm) and H-9 (multiplet at 2.65 ppm) relative to the internal standard. The decomposition products were isolated by flash column chromatography with 20% ethyl acetate in hexanes as eluent, weighed, and each identified as described below. The procedure was repeated

by running the decomposition reactions in the presence of ascorbic acid (1.0 equiv). Rates of decomposition of selected artemisinins were plotted against time, and half-lives were obtained from the plots.

c) **$FeBr_2$**: The artemisinin (1.1–1.4 mmol) and anhydrous $FeBr_2$ (0.5 equiv) in THF under N_2 were allowed to stir for 45 min at room temperature. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (100 mL). The aqueous layer was extracted with ethyl acetate (3×30 mL). The organic layer was separated and washed with saturated aqueous NH_4Cl (3×30 mL). The combined organic layer was dried ($MgSO_4$). After filtration and concentration of the filtrate under reduced pressure, the products were separated by chromatography with ethyl acetate/hexanes as eluent.

d) **$Fe(OAc)_2$ and 4-oxo-TEMPO**: A solution of anhydrous $Fe(OAc)_2$ (1.5 equiv) in CH_3CN (8 mL) was added to a stirred solution of artemisinin derivative (1.3–2.0 mmol) and 4-oxo-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 2.0 equiv) in CH_2Cl_2 (8 mL) under N_2 at room temperature. After 24 h, the reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (16 mL). The aqueous layer was extracted with ethyl acetate (3×32 mL). The organic layer was separated and washed with saturated aqueous NH_4Cl (2×32 mL). The combined organic layer was dried ($MgSO_4$). After filtration and concentration of the filtrate under reduced pressure, the products were separated by chromatography with ethyl acetate/hexanes as eluent.

2) SERCA assay in oocyte preparations

Assays were conducted after microinjection of cRNA as previously described.[89] Ca^{2+} ATPase activity was measured (pCa 5.5, 10 μg total protein, 25 °C) using a coupled enzyme assay as described, and K_i refers to apparent half-maximal inhibition of Ca^{2+}-dependent ATPase activity. The effects of DFO (100 μM) on inhibition of PfATP6 activity were assessed using concentrations of inhibitors that are close to their K_i values.

3) Parasite culture and isobologram analysis

Parasites (clone 3D7, D10, and W2) were cultured using standard techniques. IC_{50} values were determined within 48 h of adding antimalarials. For isobologram analyses (Figure 5), ratios fixed at 10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10 of inhibitor A to that of inhibitor B were used. The middle concentration of the inhibitors (5:5) was aimed to be the IC_{50} of each drug. The molar ratios of each drug were calculated, and the IC_{50} values of the mixtures presented in relation to the IC_{50} of the individual drugs.

4) Decomposition of artemisinins

*Artemisinin **1***:

a) **$FeSO_4$**: Compounds **1** (34.8 mg, 31%), **14** (22.2 mg, 20%), and **15** (29.8 mg, 26%) were obtained from $FeSO_4{\cdot}7H_2O$ (33.2 mg, 0.3 equiv) and **1** (112.8 mg, 0.4 mmol) in CH_3CN/deionized H_2O (1:1, 8 mL) followed by chromatography with ethyl acetate/hexanes (20:80); **14** colorless solid, mp: 92–94 °C (92–94 °C,[9] 91–92 °C[90]); ^1H NMR: $\delta = 1.00$ (d, $J = 6.2$ Hz, 3H, 6-Me), 1.04–1.16 (m, 1H), 1.22 (d, $J = 7.0$ Hz, 3H, 9-Me), 1.43–1.60 (m, 3H), 1.85–2.10 (m, 5H), 2.17 (s, 3H, MeCO), 3.13–3.22 (m, 1H), 3.95 (ddd, $J = 8.8$, 8.2, 7.0 Hz, 1H), 4.22 (dd, $J = 9.4$, 9.4 Hz, 1H), 6.63 ppm (s, 1H); **15** colorless solid, mp: 200–201 °C (199–201 °C[9], 190–192 °C[90]); ^1H NMR: $\delta = 0.96$ (d, $J = 6.2$ Hz, 3H, 6-Me), 0.98–1.19 (m, 2H), 1.22 (d, $J = 7.0$ Hz, 3H, 9-Me), 1.41–1.58 (m, 5H), 2.00 (s, 3H, 3-Me), 1.82–2.35 (m, 3H), 3.19–3.26 (m, 1H), 3.63 (s, 1H), 5.63 ppm (s, 1H, H-12).

b) **$FeSO_4$–ascorbic acid**: The above procedure was repeated with ascorbic acid (70.4 mg, 0.4 mmol, 1.0 equiv) to provide **1** (12.6 mg, 11%), **14** (35.6 mg, 32%), and **15** (35.6 mg, 32%). Other products were formed in smaller amounts, but were not characterized.

*Dihydroartemisinin **2***:

c) **$FeBr_2$**: DHA (500 mg, 1.76 mmol) and $FeBr_2$ (190 mg, 0.88 mmol, 0.5 equiv) in THF (15 mL) were stirred under a N_2 atmosphere for 45 min. Afterward, the mixture was quenched with saturated aqueous sodium hydrogen carbonate (100 mL) and then extracted with ethyl acetate (3×30 mL). The combined organic layer was then washed with saturated aqueous NH_4Cl. The aqueous layer was extracted with ethyl acetate (3×30 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure left a pale-yellow residue, which on chromatography with ethyl acetate/hexanes (10:90) gave firstly the 2-deoxyglycal **19** (9.5 mg, 2.2%) and the tricarbonyl compound **18** (238.4 mg, 57%) as an oil. No DHA was recovered. Recrystallization of the 2-deoxyglycal **19** from ethyl acetate/hexanes gave colorless bar-shaped crystals, mp: 103.5–104.5 °C; $[\alpha]_D^{22} = -13.1°$ ($c = 0.63$, $CHCl_3$); ^1H NMR: $\delta = 0.92$ (d, $J = 4.9$ Hz, 3H, 6-Me), 1.03–1.39 (m, 3H), 1.47 (s, 3H, 3-Me), 1.62 (br s, 3H, 9-Me), 1.67–1.78 (m, 2H), 1.79–1.98 (m, 3H), 2.35–2.45 (m, 1H), 5.58 (s, 1H, H-12), 6.04–6.05 ppm (q, 1H, $J = 1.2$ Hz, H-10); IR (film): $\tilde{\nu} = 2990$, 2947, 2927, 2873, 1681.9, 1555, 1496, 1457, 1392, 1359, 1322, 1269, 1230, 1212, 1162, 1141, 1121, 1083, 1034, 998, 966, 941, 901 cm^{-1}; MS (CI) calcd: 250.1569 [M^+], found: 250.1551; calcd: 251.1647 [M^++H], found: 251.1668.

2-DeoxyDHA **35**, mp: 142–144 °C, was prepared by catalytic hydrogenation of DHA as described.[2] 2-DeoxyDHA (500 mg, 1.87 mmol) in CH_2Cl_2 (10 mL) under N_2 was treated sequentially with methanesulfonyl chloride (0.35 mL, 2.24 mmol, 1.2 equiv) and triethylamine (0.39 mL, 2.80 mmol, 1.5 equiv) at room temperature. After 1.5 h, the mixture was treated with saturated NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure left a pale-yellow residue, which on chromatography with ethyl acetate/hexanes (15:85) gave the 2-deoxyglycal **19** (370 mg, 79%), identical with the sample obtained from Fe^{2+} decomposition of DHA, and a second uncharacterized dimeric product (64.8 mg, 6.7%).

The tricarbonyl compound **18** was obtained as a single epimer when it was freshly isolated: ^1H NMR: $\delta = 1.095$ (d, $J = 5.8$ Hz, 3H, 3-Me), 1.17 (d, $J = 7.3$ Hz, 3H, 1″-Me), 1.48–1.68 (m, 4H), 1.71–1.96 (m, 4H), 2.13 (s, 3H, MeCO), 2.30–2.43 (m, 1H), 2.48–2.65 (m, 2H), 2.70–2.81 (m, 1H), 9.74 ppm (s, 1H, CHO). The characterization of this compound is described elsewhere.[55]

*Artesunate **3***:

a) **$FeSO_4$**: Compounds **3** (6.7 mg, 6%), **22** (53.3 mg, 46%), and **23** (25.7 mg, 22%) were obtained from $FeSO_4{\cdot}7H_2O$ (24.9 mg, 0.3 equiv) and **3** (115.2 mg, 0.3 mmol) in CH_3CN/deionized H_2O (1:1, 6 mL) followed by chromatography with ethyl acetate/hexanes (25:75) containing 0.05% acetic acid. **22**[9] colorless oil; ^1H NMR: $\delta = 0.86$ (d, $J = 7.0$ Hz, 3H, 6-Me), 0.94 (d, $J = 6.2$ Hz, 3H, 9-Me), 1.34–1.46 (m, 1H), 1.48–1.62 (m, 2H), 1.70–1.88 (m, 3H), 1.90–2.01 (m, 3H), 2.11 (s, 3H, MeCO), 2.30–2.41 (m, 2H), 2.30–2.72 (m, 4H), 3.92 (dd, $J = 7.9$, 7.6 Hz, 1H), 4.27 (dd, $J = 9.7$, 9.4 Hz, 1H), 5.81–5.84 (d, $J = 9.7$ Hz, 1H), 6.20 ppm (s, 1H, H-12); ^{13}C NMR: $\delta = 12.28$,

20.87, 21.85, 22.88, 27.94, 28.81, 29.28, 30.79, 34.18, 35.73, 47.55, 55.19, 69.12, 80.32, 91.21, 93.68, 168.74, 170.82, 176.48 ppm; MS (ESI), *m/z* 407.1800 [M^++Na], 430.2600 [M^++2Na]; **23**[9] colorless oil; ^{1}H NMR: δ=0.91 (d, J=6.2 Hz 3H, 9-Me), 0.99 (d, J=7.3 Hz, 3H, 6-Me), 1.15–1.62 (m, 8H), 1.59 (s, 3H, 3-Me), 1.75–1.95 (m, 3H), 2.49–2.59 (m, 1H), 2.61–2.71 (m, 4H), 3.49–3.58 (m, 1H), 5.27 (s, 1H, H-12), 5.75 ppm (d, J=7.0 Hz, 1H, H-10); ^{13}C NMR: δ=15.06, 18.90, 20.88, 22.97, 28.84, 29.59, 30.67, 30.83, 34.34, 35.33, 41.00, 41.72, 69.86, 82.89, 95.60, 95.93, 108.43, 171.72, 176.04 ppm; MS (ESI), *m/z* calcd: 407.1682, found: 407.1683 [M^++Na]; calcd: 267.1596, found: 267.1587 [$M^+-(CH_2COOH)_2$].

b) **$FeSO_4$–ascorbic acid**: The above procedure was repeated with ascorbic acid (52.8 mg, 0.3 mmol, 1.0 equiv) to provide **3** (2.7 mg, 2%), **22** (64.2 mg, 56%), and **23** (25.3 mg, 22%).

*10-Deoxo-10-dihydroartemisinin **5***:

a) **$FeSO_4$**: From $FeSO_4\cdot 7H_2O$ (33.2 mg, 0.3 equiv) and **5** (107.2 mg, 0.4 mmol) in CH_3CN/deionized H_2O (1:1, 8 mL) followed by chromatography with ethyl acetate/hexanes (20:80) gave, in order of elution, **5** (3.2 mg, 3%), **26** (4.2 mg, 4%), **27** (2.2 mg, 2%), **25** (45 mg, 42%), and **24** (45 mg, 42%). **26**[54,56] colorless oil; ^{1}H NMR: δ=1.01 (d, J=6.7 Hz, 3H, 3-Me), 1.09 (d, J=6.2 Hz, 3H, 1′-Me), 1.36–1.60 (m, 3H), 1.74–2.11 (m, 5H), 2.14 (s, 3H, MeCO), 2.21–2.31 (m, 1H), 2.31–2.42 (m, 2H), 2.51–2.62 (m, 1H), 4.08 (dd, J=5.3, 5.6 Hz, 1H), 4.21 (dd, J=3.8, 3.8 Hz, 1H), 8.08 ppm (s, 1H, –OCHO); ^{13}C NMR: δ=15.54, 20.17, 20.55, 29.87, 30.18, 31.60, 34.65, 40.41, 41.25, 53.18, 56.86, 66.32, 160.87, 208.65, 211.96 ppm; MS (CI, NH_3): *m/z* 268 [M^+] (4%), 269 [MH^+] (38%), 270 [MH^+] ^{13}C, (6%); $C_{15}H_{24}O_4$ (268.4) anal. calcd: C 67.14, H 9.01, found: C 66.72, H 8.86%; **27**, colorless fine crystals, mp: 159.7–160.4 °C (158–160 °C[57]); ^{1}H NMR: δ=0.78 (d, J=7.0 Hz, 3H), 0.98 (d, J=6.2 Hz, 3H), 1.36–1.84 (m, 5H), 1.59 (s, 3H), 1.87–1.96 (m, 3H), 2.04–2.18 (m, 3H), 3.38 (dd, J=11.7, 11.7 Hz, 1H), 3.73 (dd, J=5.9, 5.6 Hz, 1H), 4.09–4.12 (m, 1H), 4.91 ppm (s, 1H); **25**[9] colorless square prisms, mp: 193.7–194.6 °C; ^{1}H NMR: δ=0.89 (d, J=9.7 Hz, 3H), 0.91–0.93 (d, J=7.3 Hz, 3H), 1.00–1.05 (m, 1H), 1.56 (s, 3H), 1.21–1.60 (m, 5H), 1.71–1.81 (m, 2H), 1.86–1.99 (m, 2H), 2.24–2.35 (m, 1H), 3.30–3.35 (dd, J=5.0, 5.0 Hz, 1H), 3.54–3.55 (m, 1H), 3.89 (dd, J=6.6, 5.7 Hz 1H), 5.18 ppm (s, 1H); ^{13}C NMR: δ=16.64, 19.07, 20.97, 24.12, 26.77, 30.71, 34.71, 35.37, 40.15, 42.15, 64.97, 70.08, 83.55, 95.82, 107.60 ppm; IR (film): $\tilde{\nu}$=722, 818, 879, 932, 954, 980, 1019, 1030, 1044, 1058, 1084, 1107, 1151, 1226, 1263, 1282, 1374, 1386, 1455, 1734, 2842, 2872, 2881, 2945, 2956, 2999, 3535 cm^{-1}; MS (CI, CH_4) *m/z* 154 (30%), 208 (11%), 251 (100%), 252 (18%), 268 [M^+] (4%), 269 [M^++H] (36%); **24**[9] colorless crystals, mp: 93.4–94.4 °C; ^{1}H NMR: δ=0.725 (d, J=7.2 Hz, 3H), 0.96 (d, J=6.0 Hz, 3H), 0.85–1.09 (m, 1H), 1.33–1.40 (m, 1H), 1.49–1.86 (m, 5H), 1.91–2.00 (m, 2H), 2.12 (s, 3H), 2.40–2.51 (m, 1H), 3.54 (dd, J=5.9, 5.9 Hz, 1H), 3.72 (ddd, J=5.1, 5.1, 5.1 Hz, 1H), 3.92 (dd, J=8.3, 7.5 Hz, 1H), 4.26 (dd, J=7.9, 7.9 Hz, 1H), 5.97 ppm (s, 1H); ^{13}C NMR: δ=13.07, 20.96, 21.36, 21.96, 28.06, 30.03, 30.80, 35.65, 47.44, 55.91, 68.04, 69.02, 80.68, 93.05, 169.39 ppm; MS (CI, CH_4) *m/z* 165 (6%), 209 (100%), 210 (16%), 251 (46%), 225 (15%), 267 [M^+–H] (50%), 268 [M^+] (40%).

b) **$FeSO_4$–ascorbic acid**: The above procedure was repeated with ascorbic acid (70.4 mg, 0.4 mmol, 1.0 equiv) to provide **26** (6.3 mg, 6%), **25** (43 mg, 40%), and **24** (58 mg, 54%).

c) **$FeBr_2$**: From $FeBr_2$ (21.6 mg, 0.5 equiv) and **5** (54.9 mg, 0.2 mmol) in THF (2 mL) followed by chromatography with ethyl acetate/hexanes (30:70) gave, in order of elution, **28** (4.4 mg, 9%), unreacted **5** (1.8 mg, 3%), **26** (34.7 mg, 63%), **27** (4.7 mg, 9%), and **24** (7.1 mg, 13%). **26**, colorless crystals, mp: 104–105 °C (102–104 °C[54]); ^{1}H NMR: δ=0.90 (d, J=6.0 Hz, 3H), 0.925 (d, J=7.6 Hz, 3H), 1.53 (s, 3H), 1.68–1.90 (m, 1H), 2.24–2.31 (m, 1H), 3.31 (dd, J=11.3, 4.4 Hz, 1H), 3.94–3.98 (dd, J=11.5, 6.8, Hz, 1H), 5.25 ppm (s, 1H); ^{13}C NMR: δ=16.67, 18.91, 22.14, 24.02, 24.06, 26.42, 34.47, 34.52, 35.33, 40.02, 45.88, 64.41, 82.49, 96.08, 107.14 ppm; MS (CI, NH_3): *m/z* 252 [M^+] (8%), 253 [M+H$^+$] (100%), 270 [M+NH_4^+] (72%).

d) **$Fe(OAc)_2$ and 4-oxo-TEMPO**: From $Fe(OAc)_2$ (522.0 mg, 1.5 equiv), **5** (539.2 mg, 2.0 mmol), and 4-oxo-TEMPO (681.0 mg, 2.0 equiv) in CH_3CN/CH_2Cl_2 (1:1, 16 mL) was obtained after chromatography with ethyl acetate/hexanes (30:70), in order of elution, **5** (6.6 mg, 1%), **26** (106.8 mg, 20%), **24** (128.6 mg, 24%), *N*-hydroxy-2,2,6,6-tetramethyl-1-piperidine (261.2 mg, 38%), and the radical-trapped product **42** (26.9 mg, 3%). **42**, colorless oil, ^{1}H NMR: δ=0.78 (d, J=7.3 Hz, 3H), 0.86–0.94 (m, 1H), 0.94 (d, J=6.4 Hz, 3H), 1.02–1.23 (m, 3H), 1.16 (s, 3H), 1.17 (s, 3H), 1.24–1.35 (m, 1H), 1.29 (s, 3H), 1.32 (s, 3H), 1.40–1.51 (m, 1H), 1.53–1.67 (m, 5H), 1.81–1.86 (m, 1H), 2.12–2.28 (m, 1H), 2.14 (s, 3H), 2.32–2.42 (m, 1H), 2.55–2.60 (m, 1H), 2.85 (s, 1H), 3.52 (dd, J=11.7, 11.7 Hz, 1H), 3.67–3.91 (m, 2H), 6.05 ppm (s 1H); ^{13}C NMR: δ=13.46, 21.14, 21.21, 21.87, 22.90, 23.00, 26.45, 28.84, 32.85, 33.14, 34.70, 35.76, 49.07, 51.18, 53.65, 53.70, 63.24, 63.31, 68.57, 72.85, 78.70, 92.12, 169.41, 208.21 ppm; IR (film): $\tilde{\nu}$=456, 467, 538, 601, 617, 666, 696, 736, 804, 847, 864, 919, 973, 1002, 1066, 1113, 1145, 1227, 1305, 1363, 1376, 1467, 1727, 1756, 2877, 2961, 3500 cm^{-1}; MS (CI, CH_4) *m/z* (%) 440 (3), 380 (45), 282 (52), 209 (100); MS (CI), calcd: 440.3012, found: 440.3039 [M^++H], calcd: 381.2879, found: 381.2822 [M^+–$OCOCH_3$], calcd: 380.2801, found: 380.2794 [M^+–$HOCOCH_3$].

e) **$Fe(OAc)_2$ and 4-oxo-TEMPO mixed experiment**: A solution of $Fe(OAc)_2$ (417.6 mg, 3.0 equiv), **5** (215.2 mg, 0.8 mmol), the 1,2,4-trioxolane **13**[51] (211.3 mg, 0.8 mmol), and 4-oxo-TEMPO (544.8 mg, 2.0 equiv) in CH_3CN/CH_2Cl_2 (1:1, 12 mL) under N_2 was stirred at room temperature for 24 h. It was quenched with saturated aqueous sodium hydrogen carbonate (12 mL) followed by acetic acid (3 mL). The aqueous layer was extracted with ethyl acetate (3× 24 mL). The organic layer was separated and washed with saturated aqueous NH_4Cl (2×24 mL). Filtration and concentration of the filtrate under reduced pressure gave a residue which was redissolved in ethyl acetate (2 mL). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (3×8 mL). The organic layer was separated as 'organic layer A'. The combined aqueous layer was acidified with 2 M aqueous H_2SO_4. The acidified aqueous layer was extracted with ethyl acetate (3×60 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the latter under reduced pressure gave solely **44**, a white solid (196.3 mg, 73%), with data as previously described:[51] ^{1}H NMR: δ=1.14 (s, 6H), 1.14–1.35 (m, 3H), 1.30 (s, 6H), 1.48–1.60 (m, 3H), 2.09–2.29 (m, 7H), 2.46–2.62 (m, 3H), 4.03–4.14 ppm (m, 1H). The 'organic layer A' was submitted to chromatography with ethyl acetate/hexanes (30:70) to give **5** (29.2 mg, 14%), **36** (27.3 mg, 13%), **24** (20.4 mg, 9%), *N*-hydroxy-2,2,6,6-tetramethyl-1-piperidine (80.8 mg, 15%), and the radical-trapped product **42** (11.9 mg, 3%).

f) **Thermolysis**: N_2 was slowly passed over neat crystalline **5** (100 mg, 0.37 mmol) in a round-bottom glass flask immersed in an oil bath at 100 °C during 18 h. The resulting mixture was submitted to chromatography with ethyl acetate/hexanes (30:70) to give **5** (17.4 mg, 17%) and **26** (56.2 mg, 56%). Heating for longer periods resulted in formation of more complex product mixtures, evidently arising from subsequent reactions of **26**.

*10-(4′-Fluorophenyl)-10-deoxy-10-dihydroartemisinin **6***:

a) **$FeSO_4$**: $FeSO_4\cdot 7H_2O$ (24.9 mg, 0.3 equiv) in deionized H_2O (3 mL) was added dropwise to a stirred solution of **6** (108.6 mg, 0.3 mmol)

in CH_3CN (3 mL). The eluent for chromatography was ethyl acetate/hexanes (20:80), which returned **6** (194 mg, 96%), **29** (~1 mg, <1%), **31** (2.1 mg, 2%) and **30** (1 mg, 1%). **29** colorless oil, ^{1}H NMR: $\delta=0.44$ (d, $J=7.5$ Hz, 3H), 0.99 (d, $J=6.3$ Hz, 3H), 1.21–1.55 (m, 5H), 1.59–2.04 (m, 3H), 2.06 (s, 3H), 2.28–2.38 (m, 1H), 2.66–2.74 (m, 1H), 3.83–3.90 (m, 1H), 4.10–4.16 (m, 1H), 5.52 (d, $J=6.6$ Hz, 1H, H-10), 6.26 (s, 1H, H-12), 6.94–7.02 (m, 2H, 2×Ph-H), 7.20–7.27 ppm (m, 2H, 2×Ph-H); ^{13}C NMR: $\delta=12.87$, 20.80, 22.13, 27.67, 31.51, 33.14, 35.56, 37.92, 39.64, 45.35, 53.13, 55.50, 57.34, 68.78, 72.93, 81.96, 92.51, 102.65, 114.82 (d, $J_{CF}=20.93$ Hz, Ph), 127.61 ppm (d, $J_{CF}=7.73$ Hz, Ph); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta=-116.76$ ppm; MS (CI, CH_4): *m/z* 275 (100%), 317 (81%), 361 [M^+-H] (19%), 391 (23%); MS (CI), *m/z* (%) calcd: 362.1893, found: 362.1866 [M^++H] (25), calcd: 361.1815, found: 361.1830 [M^+] (100); **31**, colorless oil, ^{1}H NMR: $\delta=1.00$ (d, $J=6.6$ Hz, 3H), 1.03 (d, $J=6.0$ Hz, 3H), 1.37–1.60 (m, 5H), 1.73–1.88 (m, 5H), 2.15 (s, 3H), 2.22–2.57 (m, 1H), 2.50–2.61 (m, 1H), 6.07 (d, $J=6.0$ Hz, 1H, H-10), 6.96–7.02 (m, 2H, 2×Ph-H), 7.08–7.22 (m, 2H, 2×Ph-H), 8.10 ppm (s, 1H, CHO); ^{13}C NMR: $\delta=12.29$, 20.47, 20.85, 30.06, 30.24, 31.49, 35.01, 39.64, 40.66, 41.67, 53.01, 57.32, 76.55, 115.57 (d, $J_{CF}=21.45$ Hz, Ph), 128.16 (d, $J_{CF}=7.95$ Hz, Ph), 135.55 (d, $J_{CF}=3.15$ Hz, Ph), 160.57 (d, $J_{CF}=19.73$ Hz, Ph), 163.96, 208.98, 212.54 ppm; ^{19}F NMR (300 MHz, $CDCl_3$): $\delta=-114.42$ ppm; MS (CI, CH_4): *m/z* 182 (26%), 317 (100%), 318 (32%), 357 (24%), 361 [M^+-H] (15%), 391 (8%); MS (CI), *m/z* (%) calcd: 316.1839, found: 316.1838 [$M^+-HCOOH_2$] (25); calcd: 317.1917, found: 317.1917 [M^+-HCOOH] (100); **30**, colorless oil, ^{1}H NMR: $\delta=0.44$ (d, $J=7.8$ Hz, 3H), 0.92 (d, $J=6.0$ Hz, 3H), 0.98–1.07 (m, 1H), 1.25–1.57 (m, 4H), 1.61 (s, 3H), 1.72–1.84 (m, 3H), 1.96–2.15 (m, 3H), 2.48–2.56 (m, 1H), 3.59 (m, 1H), 5.22 (d, $J=7.5$ Hz, 1H, H-10), 5.38 (s, 1H, H-12), 6.95–7.01 (m, 2H, 2×Ph-H), 7.15–7.20 ppm (m, 2H, 2×Ph-H); ^{13}C NMR: $\delta=14.66$, 19.01, 20.76, 25.51, 30.09, 30.87, 31.13, 34.80, 35.72, 38.14, 40.58, 41.58, 52.61, 70.15, 71.08, 83.34, 97.14, 107.48, 114.88 (d, $J_{CF}=21.15$ Hz, Ph), 128.15 ppm (d, $J_{CF}=8.03$ Hz, Ph); ^{19}F NMR (282 MHz, $CDCl_3$): $\delta=-116.46$ ppm; MS (CI, CH_4): *m/z* (%)=391 (8), 363 (54), 345 (53), 317 (62), 267 (100).

b) **$FeSO_4$–ascorbic acid**: The above procedure was repeated with ascorbic acid (52.8 mg, 0.3 mmol, 1.0 equiv) to provide **6** (190 mg, 94%), **29** (~1 mg, <1%), and **30** (4 mg, 4%).

c) **$FeBr_2$**: From $FeBr_2$ (21.6 mg, 0.5 equiv) and **6** (73.5 mg, 0.2 mmol) in THF (2 mL) after chromatography with ethyl acetate/hexanes (20:80) gave, in order of elution, **29** (10.2 mg, 14%), **31** (12.4 mg, 17%), and another unidentified product (3.3 mg, 5%) as a colorless oil: ^{1}H NMR: $\delta=1.00$ (d, $J=1.5$ Hz, 3H), 1.02 (d, $J=8.2$ Hz, 3H), 1.18–1.31 (m, 1H), 1.45–1.65 (m, 6H), 1.78–1.86 (m, 1H), 2.08–2.31 (m, 2H), 2.10 (s, 3H), 2.48–2.65 (m, 1H), 4.52 (d, $J=9.7$ Hz, 1H), 7.00–7.06 (m, 2H), 7.25–7.30 ppm (m, 2H); MS (CI, CH_4): *m/z* (%) 317 (100), 259 (46), 180 (42); MS (ESI), *m/z* calcd: 317.1917, found: 317.1922 [M^++H].

Artemisone **7**:

a) **$FeSO_4$**: From $FeSO_4\cdot7H_2O$ (24.9 mg, 0.3 equiv) and **7** (120.3 mg, 0.3 mmol) in CH_3CN/deionized H_2O (1:1, 6 mL) followed by chromatography with ethyl acetate/hexanes (50:50) gave, in order of elution, **20** (10.7 mg, 16%), **18**[9] (3.4 mg, 5%), unreacted **7** (17.4 mg, 14%), and **33** (34 mg, 28%). **20**[9] colorless oil; ^{1}H NMR: $\delta=0.95$ (d, $J=6.0$ Hz, 3H), 1.17 (d, $J=7.2$ Hz, 3H), 1.44–1.50 (m, 2H), 1.66–1.77 (m, 3H), 1.92–2.13 (m, 3H), 2.39–2.43 (m, 2H), 3.93–3.97 (m, 1H), 4.10–4.14 (m, 1H), 9.55–9.56 (d, $J=2.4$ Hz, 1H), 9.94 (s, 1H); **18**[55] pale-yellow viscous oil; ^{1}H NMR: $\delta=1.09$ (d, $J=7.0$ Hz, 3H), 1.48–1.65 (m, 1H), 1.69–1.82 (m, 1H), 1.84–1.94 (m, 1H), 2.01–2.18 (m, 1H), 2.13 (s, 3H), 2.30–2.43 (m, 1H), 2.50–2.62 (m, 1H), 2.67–2.81 (m, 1H), 9.73 ppm (s, 1H); **33** colorless oil; ^{1}H NMR: $\delta=0.84$ (d, $J=7.0$ Hz, 3H, 9-Me), 0.89 (d, $J=6.2$ Hz, 3H, 6-Me), 0.95–0.97 (m, 1H), 1.05–1.30 (m, 3H), 1.37–1.62 (m, 2H), 1.52 (s, 3H, 3-Me), 1.77–1.96 (m, 2H), 2.12–2.33 (m, 2H), 2.44–2.54 (m, 1H), 3.00–3.18 (m, 4H), 3.24–3.42 (m, 4H), 3.55 (d, $J=2.6$ Hz, 1H), 4.21–4.25 (d, $J=10.3$ Hz, 1H, H-10), 5.28 ppm (s, 1H, H-12); ^{13}C NMR: $\delta=14.59$, 19.13, 21.68, 22.84, 28.29, 30.64, 34.59, 35.29, 42.32, 42.46, 47.01, 52.37, 69.77, 83.62, 93.49, 96.01, 108.39 ppm; IR (film): $\tilde{\nu}=451$, 467, 515, 535, 667, 734, 799, 811, 855, 875, 921, 944, 979, 1016, 1077, 1125, 1220, 1275, 1305, 1383, 1453, 1645, 2875, 2932, 3500 cm^{-1}; MS (CI, CH_4): *m/z* (%) 402 (100), 267 (75), 175 (79); MS (ESI), *m/z* calcd: 402.1950, found: 402.1951 [M^++H], calcd: 267.1596, found: 267.1604 [M^+-4-(*S*,*S*-dioxo)thiomorpholin-1-yl].

b) **$FeSO_4$–ascorbic acid**: Repetition of the above procedure with ascorbic acid (52.8 mg, 0.3 mmol, 1.0 equiv) gave **20** (9.4 mg, 14%), **18** (4.4 mg, 6%), **7** (4.2 mg, 3%), and **33** (24.2 mg, 20%).

c) **$FeBr_2$**: Compounds **18** (64.6 mg, 22%), **20** (80.4 mg, 29%), and **34** (41.7 mg, 9%) were obtained from $FeBr_2$ (269.6 mg, 0.5 equiv) and **7** (501.3 mg, 1.25 mmol) in THF (12 mL) after chromatography with ethyl acetate/hexanes (40:60). **34** colorless rectangular plates, mp: 192–193 °C; $[\alpha]_D^{22}=-126$ ($c=0.66$, $CHCl_3$); ^{1}H NMR: $\delta=0.85$ (d, $J=7.0$ Hz, 3H, 9-Me), 0.92 (d, $J=5.9$ Hz, 3H, 6-Me), 1.01–1.38 (m, 5H), 1.49 (s, 3H, 3-Me), 1.52–1.61 (m, 2H), 1.66–1.73 (m, 2H), 1.75–1.90 (m, 2H), 2.43–2.55 (m, 1H), 3.01–3.21 (m, 4H), 3.26–3.42 (m, 4H), 4.21–4.25 (d, $J=10.6$ Hz, 1H, H-10), 5.32 ppm (s, 1H, H-12); ^{13}C NMR: $\delta=14.84$, 19.32, 22.41, 22.81, 25.12, 28.21, 34.66, 34.87, 35.60, 42.71, 46.56, 47.12, 52.38, 82.90, 93.77, 96.80, 108.49 ppm; IR (film): $\tilde{\nu}=414$, 428, 432, 445, 453, 471, 872, 973, 1009, 1073, 1125, 1141, 1206, 1277, 1304, 1383, 2873, 2929 cm^{-1}; MS (CI), *m/z* (%) calcd: 386.2001, found: 386.2005 [M^++H] (100).

d) **$Fe(OAc)_2$ and 4-oxo-TEMPO**: From $Fe(OAc)_2$ (339.3 mg, 1.5 equiv), **7** (521.9 mg, 1.3 mmol), and 4-oxo-TEMPO (442.7 mg, 2.0 equiv) in CH_3CN/CH_2Cl_2 (1:1, 16 mL) was obtained after chromatography with ethyl acetate/hexanes (50:50), in order of elution, **32** (60.0 mg, 12%), **20** (31.6 mg, 11%), **33** (40.0 mg, 8%), **18** (10.1 mg, 3%), **43** (69 mg, 10%), and *N*-hydroxy-2,2,6,6-tetramethyl-1-piperidine (303 mg, 7%). **32** colorless plates, mp: 120.4–121.2 °C; ^{1}H NMR: $\delta=0.80$ (d, $J=7.3$ Hz, 3H), 0.94 (d, $J=6.4$ Hz, 3H), 0.99–1.09 (m, 1H), 1.24–1.30 (m, 1H), 1.32–1.40 (m, 1H), 1.44–1.62 (m, 2H), 1.73–1.86 (m, 2H), 1.90–1.97 (m, 2H), 2.12 (s, 3H), 2.32–2.42 (m, 1H), 2.98–3.15 (m, 4H), 3.30–3.45 (m, 4H), 3.84–3.92 (m, 1H), 4.19–4.24 (dd, $J=7.6$, 7.6 Hz, 1H), 4.29–4.32 (d, $J=10.5$ Hz, 1H), 5.93 ppm (s, 1H); ^{13}C NMR: $\delta=13.41$, 20.88, 21.95, 22.55, 27.92, 30.89, 31.86, 35.76, 47.13, 48.03, 52.39, 55.21, 68.88, 80.11, 92.35, 93.69, 169.60 ppm; IR (film): $\tilde{\nu}=450$, 466, 471, 595, 666, 734, 793, 857, 879, 917, 946, 998, 1033, 1080, 1125, 1227, 1272, 1306, 1368, 1456, 1639, 1752, 2933 cm^{-1}; MS (ESI) *m/z* calcd: 402.1950, found: 402.1983 [M^++H], calcd: 267.1596, found: 267.1622 [M^+-4′-(*S*,*S*-dioxo)thiomorpholin-1′-yl]; **43** colorless foam; $[\alpha]_D^{22}=-17.5°$ ($c=0.7$, $CHCl_3$); ^{1}H NMR: $\delta=0.84$–0.86 (d, $J=7.3$ Hz, 3H, 9-Me), 1.02–1.05 (d, $J=6.2$ Hz, 3H, 6-Me), 1.11 (s, 3H, Me, 4″-oxotetramethylpiperidinyl), 1.13 (s, 3H, Me, 4″-oxotetramethylpiperidinyl), 1.27 (s, 3H, Me, oxotetramethylpiperidinyl), 1.31 (s, 3H, Me, 4″-oxotetramethylpiperidinyl), 1.38–1.41 (m, 1H, H-8a), 1.42–1.52 (m, 2H, H-5), 1.58–2.10 (m, 7H, H-8, H-5a, H-6, H-7, H-9), 2.15–2.22 (m, 2H, CH_2), 2.56–2.61 (m, 2H, CH_2), 3.10–3.11 (m, 4H, H2′, 4′-(*S*,*S*-dioxo)thiomorpholin-1′-yl), 3.31–3.34 (m, 4H, H3′, 4′-(*S*,*S*-dioxo)thiomorpholin-1′-yl), 3.80–3.88 (m, 1H, OCH_2), 3.93–4.0 (m, 1H, OCH_2), 4.63–4.64 (d, $J=3.5$ Hz, 1H, H-10), 10.07 ppm (s, 1H, CHO); ^{13}C NMR: $\delta=15.43$ (C9-Me), 20.80 (C6-Me), 21.59 (C8/C7/C5), 22.68 (Me, 4″-oxotetramethylpiperidinyl), 22.78 (Me, 4″-oxotetramethylpiperidinyl), 28.63 (C5/C7/C8), 32.86 (Me, 4″-oxotetramethylpiperidinyl), 33.0 (Me, 4″-oxote-

tramethylpiperidinyl), 34.46 (C6/C9), 36.44 (C7/C5/C8), 38.48 (C9/C6), 47.07 (C2′, 4′-(*S*,*S*-dioxo)-thiomorpholin-1′-yl), 50.69 (C8a/C5a), 52.28 (C3′, 4′-(*S*,*S*-dioxo)thiomorpholin-1′-yl), 53.53 (C5a/C8a), 53.64 (2×C, CH_2, 4′′-oxotetramethylpiperidinyl), 63.09 (CMe_2, 4′′-oxotetramethylpiperidinyl), 63.16 (CMe_2, 4′′-oxotetramethylpiperidinyl), 76.73 (OCH_2), 90.42 (C12a), 103.78 (C10), 207.26 (C12), 208.62 ppm (C=O, 4′′-oxotetramethylpiperidinyl); IR (film): $\tilde{\nu}$ = 457, 463, 471, 557, 666, 707, 736, 782, 803, 857, 904, 993, 1031, 1081, 1126, 1149, 1194, 1230, 1272, 1306, 1363, 1378, 1414, 1455, 1722, 2877, 2932 cm^{-1}; MS (CI) calcd: 513.2998 [M^++H], found: 513.2997.

10-(4′-Benzyl-1′-piperazinyl)-10-deoxo-10-dihydroartemisinin ***8***:

a) **$FeSO_4$**: Unchanged compound **8** (42 mg, 96%) and products **20** (3%), and **18** (~0.5 mg, 1%) were obtained from $FeSO_4 \cdot 7H_2O$ (8.3 mg, 0.3 equiv) and **8** (44.2 mg, 0.1 mmol) in CH_3CN/deionized H_2O (1:1, 3 mL) followed by chromatography with ethyl acetate/hexanes (40:60).

b) **$FeSO_4$–ascorbic acid**: Repetition of the above procedure with ascorbic acid (17.6 mg, 0.1 mmol, 1.0 equiv) gave, after column chromatography with ethyl acetate/hexanes (40:60), **8** (12.7 mg, 29%), **20** (6.0 mg, 27%), and **18** (2.8 mg, 12%).

c) **$FeBr_2$**: From $FeBr_2$ (118.6 mg, 0.5 equiv) and **8** (486.2 mg, 1.10 mmol) in THF (12 mL) was obtained after chromatography with ethyl acetate/hexanes (40:60) **18** (12.5 mg, 5%), **20** (64.5 mg, 26%), and **35** (70 mg, 24%). **35** white needles, mp: 142–144 °C (mp: 142–143 °C[2]); ^{1}H NMR: δ = 0.90 (d, J = 5.9 Hz, 3H, 9-Me), 1.01 (d, J = 7.3 Hz, 3H, 6-Me), 1.14–1.32 (m, 5H), 1.54 (s, 3H, 3-Me), 1.48–1.60 (m, 1H), 1.68–1.75 (m, 3H), 1.77–1.88 (m, 2H), 2.16–2.26 (m, 1H), 2.71 (d, J = 7.3 Hz, 1H), 4.78 (d, J = 6.5 Hz, 1H, H-10), 5.34 ppm (s, 1H, H-12); MS (CI, NH_3) *m/z* 251 [M^+−OH] (100%), 267 [M^+−H] (46%). The compound was identical to a sample prepared by catalytic hydrogenation of DHA according to the literature procedure.[2]

10-[4′-(2′′-Pyrimidyl)-1′-piperazinyl]-10-deoxo-10-dihydroartemisinin ***9***:

a) **$FeSO_4$**: Unchanged compound **9** (42 mg, 97%) and the products **20** (~0.5 mg, 2%) and **18** (~0.3 mg, 1%) were obtained from $FeSO_4 \cdot 7H_2O$ (8.3 mg, 0.3 equiv) and **9** (43.0 mg, 0.1 mmol) in CH_3CN/deionized H_2O (1:1, 3 mL) followed by chromatography with ethyl acetate/hexanes (40:60).

b) **$FeSO_4$–ascorbic acid**: Repetition of the above procedure with ascorbic acid (17.6 mg, 0.1 mmol, 1.0 equiv) gave, after chromatography with ethyl acetate/hexanes (40:60), unchanged **9** (29.2 mg, 68%) and the products **20** (2.8 mg, 12%) and **18** (0.6 mg, 2.5%).

10-(Sulfamido)-10-deoxo-10-dihydroartemisinin ***10***:

a) **$FeSO_4$**: From $FeSO_4 \cdot 7H_2O$ (24.9 mg, 0.3 equiv) and **10** (108.6 mg, 0.3 mmol) in CH_3CN/deionized H_2O (1:1, 6 mL) in CH_3CN (3 mL) followed by chromatography with ethyl acetate/hexanes (40:60) was obtained, in order of elution, unreacted **10** (52 mg, 48%), **18** (1.3 mg, 2%), and **36** (9 mg, 8%). **36** pale-yellow oil; ^{1}H NMR: δ = 0.94–0.96 (d, 3H, 9-Me, J = 7.0 Hz), 0.98 (d, J = 5.9 Hz, 3H, 6-Me), 1.23–1.39 (m, 3H), 1.40 (s, 3H, 3-Me), 1.55–1.83 (m, 5H), 1.87–1.96 (m, 1H), 2.00–2.08 (m, 1H), 2.32–2.45 (m, 1H), 3.73–3.76 (m, 1H), 4.82 (dd, J = 10.3, 10.3 Hz, 1H, H-10), 4.87 (s, 2H, NH_2), 5.15–5.18 (d, J = 10.6 Hz, 1H, NH), 5.38 ppm (s, 1H, H-12); IR (film): $\tilde{\nu}$ = 827, 882, 926, 1006, 1030, 1052, 1098, 1159, 1356, 1458, 1637, 2927, 3283, 3366, 3567 cm^{-1}; MS (ESI), *m/z* (%) calcd: 629.3108, found: 629.2918 [2M^++H)], calcd: 267.1596, found: 267.1613 [M^+−SO_2-$(NH_2)_2$].

c) **$FeBr_2$**: Compound **18** (107.3 mg, 32%) was obtained from $FeBr_2$ (151.2 mg, 0.5 equiv) and **9** (506.8 mg, 1.40 mmol) in THF (12 mL) after chromatography with ethyl acetate/hexanes (40:60). The reaction was repeated with $FeBr_2$ (15.5 mg, 0.2 equiv) and **10** (109.8 mg, 0.3 mmol) in THF (3 mL). The mixture was quenched with brine and worked up according to the general method to leave a residue, a solution which in chloroform deposited colorless needles of sulfamide (18.4 mg, 63%), mp: 88.1–89.1 °C; ^{1}H NMR ([D_6]acetone): δ = 1.95–2.15 ppm (m, 4H, -NH_2); MS (CI, CH_4) *m/z* (%) 80 (18) [M^+−NH_2], 96 (22) [M^+], 97 (100) [MH$^+$], identical with data from an authentic sample (Aldrich).

10-(4′-Fluorophenylamino)-10-deoxo-10-dihydroartemisinin ***11***:

a) **$FeSO_4$**: Compounds **11** (68.6 mg, 61%) and **37** (30.8 mg, 27%) were obtained from $FeSO_4 \cdot 7H_2O$ (24.9 mg, 0.3 equiv) and **11** (113.1 mg, 0.3 mmol) in deionized H_2O/CH_3CN (1:1, 6 mL) after chromatography with ethyl acetate/hexanes (30:70). **37** pale-yellow oil; $[\alpha]_D^{22} = -138$ (c = 1.97, $CHCl_3$); ^{1}H NMR: δ = 0.89 (d, J = 6.4 Hz, 3H, 9-Me), 1.02 (d, J = 7.0 Hz, 3H, 6-Me), 1.19–1.35 (m, 1H), 1.40–1.51 (m, 2H), 1.55 (s, 3H, 3-Me), 1.71–1.98 (m, 5H), 2.01–2.12 (m, 1H), 2.27–2.37 (m, 1H), 2.50–2.63 (m, 1H), 3.49 (s, NH), 3.58 (s, 1H), 4.75–4.78 (d, J = 7.9 Hz, 1H, H-10), 5.27 (s, 1H, H-12), 6.66–6.70 (m, 2H, ArH), 6.84–6.92 ppm (m, 2H, ArH); IR (film): $\tilde{\nu}$ = 544, 737, 824, 885, 920, 975, 1014, 1080, 1147, 1219, 1271, 1385, 1456, 1515, 1614, 1705, 2931, 3428 cm^{-1}; MS (CI, CH_4) *m/z* (%) 111 (4), 151 (62), 179 (6), 249 (14), 267 (62), 267 (12), 360 (30), 377 (100) [M^+], 378 (92) [M^++H]; MS (ESI), *m/z* calcd: 378.2081, found: 378.2058 [M^++H], calcd: 267.1596, found: 267.1596 [M^+−NH(C_6H_6)F].

c) **$FeBr_2$**: Compounds **18** (75.4 mg, 24%) and **38** (53.7 mg, 12%) were obtained from $FeBr_2$ (140.2 mg, 0.5 equiv) and **11** (490.1 mg, 1.30 mmol) in THF (12 mL) after chromatography with ethyl acetate/hexanes (30:70). **38** pale-yellow–orange oil; $[\alpha]_D^{22} = -445$ (c = 1.24, $CHCl_3$); ^{1}H NMR: δ = 0.89 (d, J = 5.9 Hz, 3H, 6-Me), 1.04–1.28 (m, 5H), 1.42 (s, 3H, 3-Me), 1.58–1.72 (m, 4H), 1.81 (d, J = 1.5 Hz, 3H, 9-Me), 1.84–1.92 (m, 1H), 1.97–2.03 (m, 1H), 5.18 (d, J = 1.2 Hz, 1H, H-12), 5.89 (br s, 1H, H-10), 6.94–6.95 ppm (m, 4H, ArH); ^{13}C NMR: δ = 18.95 (C14), 19.64 (C15), 22.10 (C7), 24.84 (C13), 26.67 (C8), 34.64 (C5), 35.47 (C6), 35.49 (C4), 43.25 (C5a), 46.87 (C8a), 85.19 (C12), 87.90 (C12a), 107.01 (C3), 115.74 (d, $J_{C,F}$ = 22.2 Hz, ArC3′), 116.39 (d, $J_{C,F}$ = 7.5 Hz, ArC2′), 119.42 (C9), 120.81 (C10), 142.70 (ArC1′), 157.45 ppm (d, $J_{C,F}$ = 237.5 Hz, ArC4′); IR (film): $\tilde{\nu}$ = 460, 463, 467, 471, 475, 479, 783, 823, 867, 963, 1186, 1206, 1223, 1384, 1403, 1509, 2873, 2927 cm^{-1}; MS (CI, NH_3): *m/z* (%) 344 (84) [MH$^+$], 343 (100) [M^+]; MS (ESI), *m/z* 344.2103 [M^++H].

The enamine **38** (326.9 mg, 0.95 mmol) and sodium periodate (835.8 mg, 4.1 equiv) were stirred in CCl_4 (4 mL). CH_3CN (4 mL) was added followed by H_2O (6 mL). The resulting mixture was stirred at 0 °C while ruthenium(III) chloride hydrate (4.4 mg, 0.022 equiv) was added slowly. After 3 h, the reaction mixture was poured into CH_2Cl_2 (20 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3×15 mL). The combined organic layer was dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure gave a deep-brown residue that on chromatography with ethyl acetate/hexanes (40:60) gave the product **41** as a pale-yellow gum (22.0 mg, 6%); $[\alpha]_D^{22} = -128$ (c = 2.40, $CHCl_3$); ^{1}H NMR: δ = 0.96 (d, J = 5.6 Hz, 3H, 6-Me), 1.13–1.32 (m, 5H), 1.40 (s, 3H, 3-Me), 1.52–1.74 (m, 2H), 1.59 (s, 3H, 9-Me), 1.80–1.84 (m, 1H), 1.87–1.95 (m, 1H), 1.98–2.05 (m, 1H), 2.24–2.30 (m, 1H), 5.35 (s, 1H, H-12), 5.54 (br s, 1H, 9-OH), 7.07–7.13 (m, 2H, ArH), 7.19–7.24 ppm (m, 2H, ArH); ^{13}C NMR: δ = 18.88 (C14), 22.17 (C7), 23.86 (C15), 25.07 (C13), 26.52 (C8), 33.74 (C5), 34.43 (C4), 35.88 (C6), 46.02 (C5a), 47.27 (C8a), 73.38 (C9), 84.80 (C12a), 88.24 (C12), 108.77 (C3),

116.46 (d, $J_{C,F}=22.5$ Hz, ArC3′), 130.43 (C2′, $J_{C,F}=8.6$ Hz, ArC2′), 136.00 (d, $J_{C,F}=3.2$ Hz, C1′), 162.04 (d, $J_{C,F}=246.4$ Hz, ArC4′), 170.39 ppm (C10); IR (KBr): $\tilde{\nu}=542$, 816, 864, 955, 996, 1020, 1141, 1147, 1215, 1233, 1417, 1509, 1677, 2882, 2923, 2933, 2952, 3422 cm^{-1} (H-bonded OH); MS (CI), *m/z* calcd: 376.1924, found: 376.1941 [M^++H] (100).

d) **$Fe(OAc)_2$ and 4-oxo-TEMPO**: From $Fe(OAc)_2$ (391.5 mg, 1.5 equiv), **11** (567.2 mg, 1.5 mmol), and 4-oxo-TEMPO (510.5 mg, 2.0 equiv) in CH_3CN/CH_2Cl_2 (1:1, 16 mL) was obtained, after chromatography with ethyl acetate/hexanes (30:70) in order of elution, **11** (346.6 mg, 61%), **37** (13.8 mg, 2%), **38** (3.4 mg, 1%), and *N*-hydroxy-2,2,6,6-tetramethyl-1-piperidine (328.4 mg, 64%).

10-(4′-Fluorobenzylamino)-10-deoxo-10-dihydroartemisinin ***12***:

c) **$FeBr_2$**: Compounds **18** (23 mg, 8%), **40** (110.5 mg, 24%), and **39** (23.9 mg, 5%) were obtained from $FeBr_2$ (138.3 mg, 0.5 equiv) and **12** (500.5 mg, 1.28 mmol) in THF (12 mL) after chromatography with ethyl acetate/hexanes (30:70). **40** pale-orange oil; $[\alpha]_D^{22}=-116$ ($c=1.39$, $CHCl_3$); ^{1}H NMR: $\delta=0.87$ (d, $J=5.6$ Hz, 3H, 6-Me), 1.00–1.39 (m, 5H), 1.42 (s, 3H, 3-Me), 1.65 (d, $J=1.2$ Hz, 3H, 9-Me), 1.50–1.81 (m, 4H), 1.87–1.93 (m, 1H), 2.22–2.32 (m, 1H), 4.00 (d, $J=15.2$ Hz, 1H, ArCH), 4.26 (d, $J=15.2$ Hz, 1H, ArCH), 4.91 (s, 1H, H-12), 5.48 (br s, 1H, H-10), 6.95–7.01 (m, 2H, ArH), 7.21–7.26 ppm (m, 2H, ArH); ^{13}C NMR: $\delta=19.07$ (C14), 19.11 (C15), 21.91 (C7), 24.84 (C13), 25.87 (C8), 34.91 (C5), 35.57 (C4, C6), 43.10 (C5a), 46.97 (C8a), 53.55 (ArCH), 85.44 (C12), 85.61 (C12a), 106.19 (C3), 108.98 (C9), 115.30 (d, $J_{C,F}=21.3$ Hz, ArC3′), 125.42 (C10), 129.47 (d, $J_{C,F}=8.1$ Hz, ArC2′), 135.46 (d, $J_{C,F}=3.2$ Hz, ArC1′), 162.02 ppm (d, $J_{C,F}=243.6$ Hz, ArC4′); IR (film): $\tilde{\nu}=464$, 467, 471, 475, 854, 878, 958, 1150, 1221, 1384, 1509, 1604, 1682, 2874, 2926 cm^{-1}; MS (CI, NH_3) *m/z* (%) 358 (56) [MH^+], 357 (100) [M^+]; MS (ESI), *m/z* (%) calcd: 358.2182, found: 358.1975 [M^++H]; **39** yellow oil; $[\alpha]_D^{22}=-84$ ($c=0.72$, $CHCl_3$); ^{1}H NMR: $\delta=0.89$ (d, $J=6.2$ Hz, 3H, 9-Me), 0.98 (d, $J=7.3$ Hz, 3H, 6-Me), 1.06–1.46 (m, 5H), 1.52 (s, 3H, 3-Me), 1.73–1.93 (m, 5H), 2.10–2.37 (m, 2H), 3.49–3.53 (m, 1H), 3.84 (d, $J=13.5$ Hz, 1H, ArCH), 4.06 (d, $J=14.1$ Hz, 1H, ArCH), 4.09 (d, 1H, H-10), 5.28 (s, 1H, H-12), 6.94–7.00 (m, 2H, ArH), 7.29–7.35 ppm (m, 2H, ArH); IR (film): $\tilde{\nu}=481$, 826, 885, 972, 1083, 1154, 1221, 1384, 1458, 1509, 1604, 1670, 2875, 2930, 3446 cm^{-1}; MS (ESI), *m/z* (%) calcd: 392.2237, found: 392.2141 [M^++H].

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